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EFFECT OF LEVEL AND SOURCE OF DIETARY FAT ON SERUM CHOLESTEROL, AND AORTIC AND CORONARY LESIONS IN SWINE

by

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INTRODUCTION

Circulatory diseases now head the list of causes of death in the present era, chiefly because of the tremendous increase in life expectancy which has been brought about by improvements in modern day life due to advances in the areas of hygiene and preventive medicine.

In the past, considerable contributions to the knowledge of atherosclerosis have come from studies of human material, and, in the future such studies will also continue to contribute valuable information on this subject. Primarily due to the chronicity of the atherosclerotic lesion and the limitations placed on the use of man for investigative research, one has to rely on the use of animal experimentation for a solution to this problem. Extension of treatment for this condition to the species which is of most concern, homo sapiens, can only be justified after extensive animal experimentation.

Investigations by Skold (1962), Downie et al. (1963) and French et al. (1963) have shown that the arterial system of swine is subject to a lesion that has many characteristics comparable to atherosclerosis in man. These investigators suggest that the pig should serve as an excellent laboratory animal for investigative purposes regarding this condition.

In recent years, no medical question has provoked more controversy than that of the role of diet in the onset of atherosclerosis. The purpose of this study was to evaluate the effect of fat source (soyoil versus tallow), protein level and level of food intake on serum cholesterol levels and plaque formation in the coronary arteries and the thoracic and abdominal aortas of swine.

REVIEW OF LITERATURE

Lesion Distribution

Numerous articles have been published describing the distribution of atherosclerotic lesions in man; however, the number of articles reporting on the distribution of atherosclerotic lesions in animals is very limited. This review will be confined to investigations comparing the distribution of lesions in swine to that of man.

One of the earlier reports on the occurrence of atherosclerosis in swine is that of Gottlieb and Lalich (1954). These workers examined more than 2000 aortas from pigs ranging from 4 months to 3 years of age and found that the principal site of fibrous connective tissue plaques was the thoracic aorta. Bragdon et al. (1957) also reported that the principal site of experimentally produced lesions in swine was in the thoracic aorta.

Skold (1962) reported on a study carried out on 50 pigs ranging from 1 to 8½ years of age. The thoracic aorta contained more areas of fatty streaking; however, the abdominal aorta was more severely affected by plaque formation. The anterior descending branch of the left coronary artery contained the most lesions of the coronary arterial system.

Downie et al. (1963) observed that the distribution of atherosclerosis is most evident initially in the thoracic aorta of swine, but as they age, the distribution, particularly of the fibrous plaques, becomes more pronounced in the abdominal aorta. These workers noted that in the coronaries it was the proximal 5 centimeters which were most heavily involved, and also that coronary artery atherosclerosis tends to become grossly detectable after aortic involvement has become extensive.

French *et al.* (1963) examined 12 pigs between 4 and 10 years of age and found that the first 1 or 2 centimeters of the right coronary artery and of the two main divisions of the left were the commonest areas to show widespread roughening. The authors observed that the most extensive changes in the aorta began just proximal to the insertion of the diaphragm.

Duff and McMillan (1951) in a review of the pathology of atherosclerosis in man stated that the aorta is the vessel in which atherosclerosis attains its earliest and most extensive expression. Lesions have been observed in the aortas of stillborn or newborn infants. These authors also state that in the earlier stage of the disease the lesions are most abundant in the thoracic part; but, as the disease progresses, lesions develop in the abdominal aorta and eventually these surpass in severity those of the thoracic aorta. Atherosclerotic lesions of the coronary arteries appear to develop later than those of the aorta and generally are first noted in the anterior descending branch of the left coronary artery near its origin; however, they state that it is not uncommon to find severe atherosclerosis of the coronary arteries in the presence of a relatively slight degree of aortic involvement.

Levene (1956) noted that atheromatous plaques were present at the junction of the left anterior descending and circumflex coronary arteries in most middle-aged human patients.

Jennings *et al.* (1961) described in detail lesions found in a 7 year-old pig. Localized intimal thickenings in the coronary arteries and in the aorta, particularly in the abdominal segment, were noted. Calcification of the lesions was common. Cerebral artery examination showed thickenings, visible microscopically, similar to those described in man.

Schwartz and Mitchell (1962) reported that the thoracic aorta in man remains relatively free of raised atherosclerotic lesions whereas the abdominal aorta is markedly affected.

Stephenson _et al_. (1962) observed that, in man, atheromatous material tends to be deposited in greatest concentration in the abdominal aorta and common iliac arteries and at the orifices of branch vessels.

In general, it would appear that the distribution of atherosclerotic lesions in swine is quite similar to that of man, in that the lesions tend to develop first in the thoracic aorta, but as the animals age the distribution, particularly of the fibrous plaques, becomes more pronounced in the abdominal aorta. The coronary arteries appear to be affected only after the aortic involvement has become extensive and the left coronary arteries tend to show the greater incidence of lesions. These observations would seem to indicate that the pig should make a good experimental animal for investigative research on atherosclerosis.

Effect of Fat Source in the Development of Atherosclerosis

Bragdon _et al_. (1957) reported the results of a 9 week experiment in which two-year-old boars were fed a basal corn-soybean diet to which either corn oil or butter was added, such that the fat source contributed approximately 40% of the caloric value of the diets. The addition of corn oil to the basal diet resulted in no significant increase in total serum cholesterol as compared with the serum cholesterol levels of the pigs fed the basal diet without added fat. The addition of butter, however, resulted in a significant increase in the total serum cholesterol levels.

Necropsy, upon termination of the experiment, revealed atherosclerotic lesions in 50% of the aortas. The lesions were confined to the thoracic aortas and the authors state that they were identical to those described by Gottlieb and Lalich (1954). No difference in incidence was observed between treatments.

Hill et al. (1957) using purified diets, studied the effects of essential fatty acid deficiencies in baby pigs by using both miniature and conventional-size pigs. The two basal diets contained 6.4% ether extract. One of the diets provided 260 mg. of total polyunsaturated acids per kilogram and the other 114 mg. per kilogram. After 8 weeks on the EFA deficient diet, approximately two-thirds of the pigs examined had gross lesions of the aorta. Lesions were found in the media and consisted of areas of calcification. No lipid deposits were evident following staining with Sudan IV. Hearts and livers were analyzed for fatty acids, and in general, the longer the animal was on the low EFA diet the lower was its content of dienoic and tetraenoic acids. Supplementation with linoleic acid increased the concentration of dienoic and tetraenoic acids in the tissues as well as completely preventing the occurrence of aortic lesions.

Peifer and Lundberg (1957) using eight-week-old miniature pigs, studied the influence of specific fatty acids on the development of atherosclerosis. Pigs were fed a fat-free diet containing 0.1% cholesterol for 2 months. At the end of this period ethyl esters of saturated fatty acids (75% palmitate and 25% myristate and laurate), oleic, linoleic or linolenic (containing 25% linoleate) were substituted for 10% of the dietary dextrose in the original diet and this treatment was continued for one month. Dietary cholesterol was increased to 0.5% at this time. Aortas from pigs receiving ethyl linolenate showed only a small amount of plaque formation. Pigs receiving ethyl linoleate had more extensive aortic deposits, while those receiving oleate or saturated esters had very extensive deposits over the entire surface of the thoracic aorta.

Moreland _et al_. (1963) using miniature pigs demonstrated that the addition of cholesterol to the diet increased both incidence and severity of the naturally occurring disease. Lesions in the cholesterol-fed animals were histologically similar to spontaneously occurring lesions, and the authors conclude that the atherosclerotic process is accelerated rather than induced by cholesterol addition to the diet.

Rowsell _et al_. (1958) made a comparison between the effect of margarine and the effect of butter. The butter and margarine were added to a basic diet at a level to contribute 40% of the dietary caloric intake. A control group was maintained on the basal diet. The animals were killed over a period of 3 to 9 months. No significant differences were noted in mean serum cholesterol levels, between the three treatments. Animals fed butter, however, had the greatest amount of aortic atherosclerosis.

Barnes _et al_. (1959a) reported on a study in which sows of 3 to 7 years of age were used to study the effect of adding olive oil, corn oil, butterfat or hydrogenated fat at a level of approximately 7%, to a typical mixed human diet. Hydrogenated fat, and particularly butterfat, resulted in higher cholesterol values than did the unsaturated vegetable fats or the low fat diet. A comparison was also made of the serum cholesterol responses in pigs receiving the mixed-food diet and a purified diet with corn oil or butterfat. With each fat it was found that the natural mixed-food diet resulted in higher cholesterol values; however, the difference between the two types of fats appeared to be approximately constant. These authors also report that pigs receiving a corn oil diet have an average α to β lipoprotein ratio of 37:63 as measured by paper electrophoresis. This ratio is similar to that reported for man and apparently is different from that of most other species.

Reiser et al. (1959), starting with miniature pigs 7-10 weeks of age, studied the effects of three rations, each qualitatively different with respect to fat and each with and without cholesterol. A basic "low fat" diet was used and the fats, myristoyl-laurin or cottonseed oil, were added at a level of 20% of the diet, replacing a similar quantity of sucrose in the basic diet. The 2% cholesterol also was added at the expense of sucrose. Protein, vitamin and mineral levels were made sufficiently high so that high caloric levels of the fatty diets would not result in an inadequate daily intake of essential nutrients. Initially, tripalmitin was used as the saturated fat; however, it was found that not over 20% was being absorbed and after a period of 5 months on the diets, a triglyceride consisting of a 1:1 mixture of myristic and lauric acids was substituted. Time on experiment was approximately 11 months. It was found that the unsaturated fat source (cottonseed oil) increased the apparent absorption of cholesterol while the saturated fat (myristoyl-laurin) depressed cholesterol absorption. Also, when cholesterol was fed with myristoyl-laurin, the absorption of the fatty acids was depressed. A summary of the results found is presented in Table 1.

Table 1. Effect of diet on degree of absorption of fatty acids and cholesterol

Diet	Fatty acids, %	Cholesterol, %
Low fat	--	--
Low fat plus 2% cholesterol	--	67
20% myristoyl-laurin	98	--
20% myristoyl-laurin plus 2% cholesterol	89	60
20% cottonseed oil	95	--
20% cottonseed oil plus 2% cholesterol	99	87

A study was also made of changes in serum lipids after meals of saturated and unsaturated fats. No visible or optically measurable lipemia was noted. The serum cholesterol levels showed no consistent pattern. These workers, amongst other things, measured the cholesterol content of the aorta and coronary arteries upon termination of the experiment. A summary of the average values obtained is presented in Table 2. Higher levels of triglycerides were noted in the plasma and in the liver of pigs on diets containing high levels of saturated fats. Examination of the coronary arteries and the aortas of pigs, upon termination of the experiment, showed little evidence of atherosclerosis in the low fat group, and the addition of 2% cholesterol resulted in no significant difference. The substitution of myristoyl-laurin for 20% sucrose in the basal diet also resulted in no significant difference. The inclusion of 2% cholesterol, however, in the saturated fat diet resulted in arteriosclerotic lesions. The aortas of swine, fed diets containing 20% cottonseed oil, were similar to those fed the low-fat diet. The inclusion of 2% cholesterol in the 20% cottonseed oil diet resulted in the most marked

Table 2. Effect of diet on tissue cholesterol content
(mg. cholesterol/gm. tissue)

Diet	Aorta			Coronary
	Arch	Thoracic	Abdominal	
Low fat	0.92	0.86	0.82	0.13
Low fat plus 2% cholesterol	0.78	0.75	0.90	0.54
20% myristoyl-laurin	0.90	0.78	1.00	0.30
20% myristoyl-laurin plus 2% cholesterol	1.30	0.87	1.73	0.88
20% cottonseed oil	0.83	0.82	0.61	0.28
20% cottonseed oil plus 2% cholesterol	1.03	0.93	1.11	1.91

atheromatosis of any group.

Rowsell *et al.* (1960) reported on a study in which a comparison was made between the effects of egg yolk and butter. Three groups of pigs were continued on experiment for periods extending from 4 to 12 months. One group of pigs was fed a low-fat diet, a second group received a diet with 33% of the calories as egg yolk, and the third group received a diet with 33% of the calories as butter. The serum cholesterol levels of the animals fed the diet supplemented with butter did not differ significantly from those of the control animals; however, the pigs fed the diet supplemented with egg yolk had significantly higher serum cholesterol values than did the animals fed the diet supplemented with butter or the control diet. The mean amount of aorta involved with atherosclerosis in the group fed the diet supplemented with egg yolk was six times that of the controls, whereas in the pigs fed the diet supplemented with butter it was three times that found in the controls.

Barnes *et al.* (1961) used adult sows (3 to 7 years old) to measure the relative effects on serum cholesterol values of natural (cis) fats and hydrogenated fat which contained isofatty acids including transisomers. Soybean oil was partially hydrogenated to give an oil with an iodine number in the range of 80 to 105. The process of hydrogenation was considered to produce some isofatty acids including transisomers. Cottonseed oil, olive oil, safflower oil and beef tallow were analyzed and then blended in pairs to give natural fat mixtures with the same fatty acid composition as the partially hydrogenated fat. The sows were fed a low-fat diet for 3 weeks and then for 3 weeks they were fed the test diet containing 40% of the total calories present as the test fat. No significant serum cholesterol responses were noted since the hydrogenated fat resulted in the same serum cholesterol response as the corresponding natural plant fat mixtures. In addition, these authors compared the effect of triolein (cis) (cis:trans = 4:1) with trielaidin (trans) (cis:trans = 1:4) and it was found that trielaidin resulted in a lower ($P < 0.05$) serum cholesterol response than did triolein.

Downie et al. (1963) as a continuation of previous work fed four groups of pigs as follows: Group A was given the basic diet with 25% of their calories as lard; Group B was given the basic diet and 25% of their calories as lard plus an amount of cholesterol equal to that found in the egg-yolk diet given to Group C; Group C was given the basic diet plus 25% of their calories as egg yolk; Group D was given the basic diet only. A summary of the mean serum cholesterol values and the mean percent total area of the aorta involved with atherosclerosis is presented in Table 3.

Table 3. Dietary effect on atherosclerosis

Group	Cholesterol (mg. percent)	Area involved (percent)
Control	114.5	16.14
Lard	134.8	21.83
Egg yolk	158.25	32.78
Lard-cholesterol	236.1	41.95

Gresham et al. (1964) raised four groups of pigs from 4.5 to 90.9 kg. live weight on a commercial diet or on semi-synthetic diets containing no fat, 10% beef tallow or 10% maize oil. Isocaloric amounts of the diets were fed daily. Sudanophilic lesions were observed in the thoracic and abdominal aortas and the coronary arteries, but there was little difference among the groups as to the extent or severity of the Sudanophilic lesions. Pigs given the no-fat diet had varying degrees of centrilobular fatty change involving chiefly an accumulation of cholesterol esters and a fall in phospholipid.

In general, it would appear that saturated fats increase serum cholesterol levels and plaque formation in the arterial system while unsaturated fats do not exhibit this characteristic; however, some reports have shown no differences between the fat sources or the degree of saturation of the fat source. Such observations as these have lead the authors to conclude that other factors must be involved.

Effect of Energy Intake on the Development of Atherosclerosis

Peifer and Lundberg (1958) reported on an experiment conducted to evaluate simultaneously the influence of total calories, fat calories and fat unsaturation on levels of cholesterol and other lipids in the blood of miniature pigs. Weanling pigs were fed diets containing 18% protein and 0.5% cholesterol, differing as follows: (1) high calorie (37% above maintenance requirements), 18% fat; (2) maintenance calorie, 18% fat; (3) high calorie (same caloric intake as 1), 5.6% fat. Each group was further divided into 2 subgroups a and b, whose fat intake was mainly beef tallow or corn oil respectively. After 8 months on experiment groups 3a and 3b continued to maintain low plasma cholesterol levels (112 vs. 111 mg.%); corresponding values for groups 1a and 1b were almost double these values; groups 2a and 2b had intermediate levels of plasma cholesterol. Fat intake was the most important factor influencing plasma cholesterol. The groups receiving corn oil tended to have lower values than did the group receiving tallow, but the effect was not consistent. No effects attributable to total calories per se were observed.

Peifer and Lundberg (1962) reared and maintained groups of miniature pigs for a period of 5 years on diets which varied in their fat content, total unsaturation of the fat, and the total available calories. Pigs fed high caloric amounts of high-fat diets, either corn oil or beef tallow, developed a transient hypercholesteremia; whereas, pigs fed low caloric amounts of these same diets, or low-fat diets at a high caloric level showed little change in their cholesterol levels. However, high levels of fat in the diet resulted in a significant reduction in the circulating endogenous polyunsaturated fatty acids (PUFA). The groups receiving large quantities of corn oil or tallow had similar patterns of endogenous polyenoic acids in their blood. The main difference between the groups was in their levels of circulating exogenous polyenoic acids. In pigs fed low-fat diets the circulating PUFA and other classes of lipids remained essentially constant throughout the experiment.

Hays et al. (1963) reported on a trial studying the effect of energy and protein levels on blood cholesterol concentration. Protein and fat sources in this trial were of plant source. A summary of the results is shown in Table 4 and suggests that total caloric intake does affect cholesterol levels.

Generally, from the limited data available, it would appear that the energy source itself is of more importance than total caloric intake in the development of atherosclerosis. Energy coming from a saturated fat source tends to result in a greater incidence of the disease than energy coming from an unsaturated fat source.

Table 4. Effect of energy and protein on serum cholesterol levels

Daily intake:				
Protein, gm.	182	363	182	363
Energy (kcal. metab. energy)	6,000	6,000	12,000	12,000
1st reproductive cycle				
Prior to breeding	142	141	140	138
Mid gestation[a,b]	122	128	135	158
2nd reproductive cycle				
Prior to breeding[c,d]	139	136	158	152
Mid gestation[e]	131	133	154	161
3rd reproductive cycle				
Prior to breeding	152	162	156	168
Mid gestation	132	145	145	159

[a]On experimental diet 108 days.

[b]Protein effect and energy effect significant ($P<0.01$).
Protein X energy interaction significant ($P< 0.05$).

[c]Following a two week lactation period all animals were fed their respective protein levels and allowed 6,000 kcals per day.

[d]Significant carryover effect of level of energy intake during the previous gestation period ($P< 0.01$).

[e]Significant energy effect ($P< 0.01$).

Effect of Protein Level on the Development of Atherosclerosis

In a second experiment reported in the paper of Barnes et al. (1959a), adult sows were fed purified diets of high protein (13.7%) or low protein (4.9%) or a protein-free diet with butter, corn oil or beef tallow added. The authors state that the low fat and three different types of fat resulted in characteristic serum cholesterol responses but present no table of values. Serum cholesterol values were not influenced by protein levels of 13.7% or 4.9% in the diets. There was no significant effect observed during a 4-week period when all of the protein was removed and corn oil was continued as the dietary fat. However, during a second 4-week period when the fat was changed to beef tallow, the authors observed a rise in serum cholesterol above that obtained with the high-protein diet. This cholesterol elevation continued even when protein at the 4.9% level was returned to the protein-free diet.

As a continuation of their previous work, Barnes et al. (1959b) studied the effect of dietary protein level changes in the young pig. Two levels of protein (16% and 9%), and two levels of dietary fat (13% and 3%), were used with pigs beginning at 8 weeks of age. The experimental period lasted for 36 weeks. The feeding of diets containing either high fat (in the form of tallow) or low protein, resulted in increased serum cholesterol levels. The cholesterol values reached a peak between the 4th and 8th week and then declined slowly towards the levels found in adult swine. Both low protein groups failed to grow well, and of these two treatments the evidence of protein malnutrition was most marked in the low-protein, high-fat group. These animals exhibited symptoms resembling kwashiorkor in human infants. The precipitous rise of serum cholesterol level during the early months of growth was exaggerated by fat in the diet and also by protein deficiency. The authors suggest that these findings demonstrate that very high serum cholesterol values with low-fat diets can occur only during the time that protein is seriously limiting in the diet.

Hays et al. (1963) reported on a trial in which sows were carried through successive reproductive cycles. Diets containing only plant sources of protein and fat were compared to those receiving a combination of plant and animal protein. The fat source provided 16% of the total metabolizable energy. Cholesterol levels were determined at two different stages: (1) just prior to breeding when sows were fed 2.27 kg. of feed per day, and (2) during late gestation when the sows were getting 1.82 kg. of feed per day. In the first instance blood was drawn immediately after feeding, and in the second it was drawn before feeding. When the blood was drawn soon after feeding, prior to breeding, sows on animal protein and fat diets had significantly higher blood cholesterol concentrations (180 vs. 164 mg./100 ml.) than those animals fed a diet containing protein and fat of plant sources. There was no significant difference due to dietary treatment when blood samples were drawn midway during gestation, although the trend was similar (113 vs. 108 mg./100 ml.). A representative sample of sows from each diet treatment was slaughtered and the vascular system examined for gross atherosclerotic lesions. Sows on animal protein and fat had an average of 29.4% of the total area of the abdominal aorta affected as opposed to 30.0% for the animals on plant protein and fat source. Coronary artery involvement was also similar (8.1% vs. 8.8%) for the two treatments.

Hays *et al.* (1963) in their study on the effect of energy and protein levels on blood cholesterol concentration in sows, of which the results are summarized in Table 4, found a significant protein effect during mid gestation of the first reproductive cycle and also a significant protein X energy interaction.

Moreland *et al.* (1963) used a 2X2 factorial arrangement with 4 to 5 month-old miniature pigs, to study the effect of a high protein diet (30%) and a low protein diet (8%), with and without 1% cholesterol. All diets were approximately isocaloric and were fed for an experimental period of six months. The feeding of cholesterol aggravated the development of atherosclerosis; however, the dietary protein levels used were found to have had no effect on the response of the animals to the cholesterol containing diets.

In general, it would appear that protein levels by themselves have little or no effect on the production of atherosclerosis.

Effect of Strain of Animal on Serum Cholesterol Values

Lewis and Page (1956) reported on a trial in which studies were made on miniature swine of the long-lean and short-fat strains, to determine any association between body build and cholesterol-lipoprotein levels. Both the long-lean strain (ectomorphic) and the short-fat strain (mesomorphic) were fed the same ration. At 14 weeks the total serum cholesterol concentrations of fat pigs were greater than those for lean pigs (149 vs. 106 mg./100 ml.), and at 34 weeks the values were 176 vs. 126 mg./100 ml. Phospholipid trends were the same: 7.3 vs. 4.9 mg./100 ml. at 14 weeks, and 8.6 vs. 6.4 mg./100 ml. at 34 weeks. Low density lipoproteins were present in greater concentrations in the fat strain than in the lean strain.

This finding of differences in lipoprotein and cholesterol concentration in two strains of pigs of similar age and on identical diets may help to explain the great range of normal values found in the heterogenous human population on varied diets and would appear to be an important factor to be taken into consideration.

Influence of Season on Serum Cholesterol Values

Heidenreich _et al_. (1962) studied seasonal influence on serum cholesterol values. Two trials were conducted, one from November to March, and the second from July to October, starting with pigs averaging 80 and 90 days respectively. Serum total cholesterol was about 152 and 150 mg./100 ml. serum at 123 and 172 days of age in January and March; about 118, 105 and 112 at 92, 126 and 182 days in July, August and October. The seasonal differences between pigs of similar age were significant ($P < 0.01$).

The influence of season may play an important role in the level of serum cholesterol; however, the experimental technique used in the above report does not seem appropriate to justify the conclusions the authors reached, since there were a number of other variables which could have contributed to the differences observed.

Hormonal Influence on Serum Cholesterol Values

Cox and Hale (1960) reported on a trial in which barrow pigs, 9-10 weeks of age, were assigned in a testosterone study to the following treatments: controls and testosterone additions of 20 mg. per day; 0 mg. to 56.8 kg. bodyweight and 20 mg. per day thereafter; 20 mg. per day to 56.8 kg. and 0 mg. thereafter; and 0, 5, 10, 15, 20, 30, 40 and 50 mg. per day at 10-day intervals. In addition a group of boars were included for comparison with barrow controls. A second study, using a stilbestrol-supplemented feed (each pig received a minimum of 2 mg. stilbestrol per day) with 5% or 10% of beef tallow, was also reported. Testosterone caused a marked reduction in serum cholesterol, whereas stilbestrol had no apparent effect. Castration resulted in hypercholesteremia. The feeding of beef tallow at 5% or 10% of the diet produced higher levels of serum cholesterol.

In general, one would expect males to have higher cholesterol levels than females and that castrates would have an intermediate level since in man, females appear comparatively immune to atherosclerosis whereas males have a high incidence. As a result of this, it has been hypothesized that in man the female sex hormone protects a person from atherosclerosis and that the male hormone accelerates the development of the condition. The above observations in swine, however, would not support this theory.

EXPERIMENTAL

These experiments were conducted to study the effects of high and low levels of energy intake, using soybean oil and tallow as main fat sources, and the effect of protein level on serum cholesterol levels and atherosclerotic lesion development in the thoracic and abdominal aortas and coronary arteries. The effect of cholesterol addition to the above fat sources on the above criteria was also studied. Stabilized edible tallow was used and the soybean oil used was degummed and is hereafter referred to as soyoil.

General Experimental Methods

The data from the experiments reported herein are on file in the Swine Nutrition Section of the Animal Science Department, Iowa State University, Ames, Iowa. Three trials were conducted and these are numbered as Swine Nutrition Experiments 6415, 6434 and 6512.

Certain management and experimental practices were common to all three experiments and these will be discussed at this point to avoid unnecessary repetition in the discussion of each individual experiment.

All of the pigs were obtained from the swine nutrition farm breeding herd. In general, the animals were crossbred consisting of Poland China, Landrace and Yorkshire breeding. Within 24 hours after birth, needle teeth were clipped, each pig was individually weighed and ear marked for identification, and each pig was injected intraperitoneally with 100 milligrams of iron, as iron dextran, to aid in the prevention of anaemia. Male pigs were castrated at 5 to 7 days of age. All pigs were weaned between 2 and 4 weeks of age and received injections of modified hog cholera virus and antiserum, and erysipelas bacterin at approximately 7 weeks of age.

The animals were sprayed periodically with Toxaphene to control mange. All pigs were fed a standard corn-soybean diet before the experiment started.

The composition and analysis of the experimental diets are shown in Appendix Tables 24 to 31.

Pigs were randomly allotted from outcome groups based on initial weight and sex to a randomized complete block experimental design. The initial starting weights of the pigs in these experiments ranged from 23.6 to 38.2 kg.

Experiments 6415 and 6434 were conducted on concrete in a building (Unit B) consisting essentially of three parts, two of which are housing accommodation with outside concrete floored pens attached, the third part contains 24 individual feeding pens with automatic water fountains installed. The outside concrete floored pens had one automatic water fountain installed in each pen, except during winter, at which time water was placed in troughs in the pens at approximately mid-day. Water was available to all pigs at time of feeding.

Experiment 6512 was conducted on concrete in four separate houses (Unit G), each one of which consists of four pens with one automatic water fountain and one self feeder per pen.

Blood samples were taken after a 16-hour fast at the start of the experiment and then biweekly during the remainder of the experiment.

Samples were withdrawn from the anterior vena cava by the method described by Carle and Dewhirst (1942).

Total serum cholesterol determinations were carried out using a Technicon Auto Analyzer and as described in the Technicon Auto Analyzer Methodology - Method File N-24 (1964). This method is based on the methods reported by the following workers: Zlatkis et al. (1953), Zak et al. (1954) and Leffler (1959). The method consists of reacting concentrated sulfuric acid and 0.05% ferric chloride in acetic acid with a 1:10 isopropanol extract of the serum and heating at 95°C to develop color. The color was read at 550 mμ in a flowcell with a 15 mm. light path.

The pigs on experiment 6415, upon termination of the experiment, were tatooed and sent to the George A. Hormel and Company packing plant at Fort Dodge, Iowa, where the necessary materials were collected. Pigs on the other two experiments, upon termination of the experiment, were tatooed and slaughtered at the Meat Laboratory, Iowa State University, Ames, Iowa, where the necessary materials were collected.

The heart with the aorta and the first few centimeters of the iliac arteries attached was carefully dissected from the carcass after the intestines, liver and stomach had been carefully removed so as to avoid damage to any of the tissues being collected.

The coronary arteries were dissected from the heart by the method described by Skold (1962).

The aorta was divided into the thoracic and abdominal portions by transection at the hiatus aorticus of the diaphragm. In these studies the abdominal aorta also included the first few centimeters of the external and internal iliac arteries.

The aorta and coronary arteries were carefully stripped of any adventitial fat and tissue and were then cut open longitudinally and kept in an open position by pressing the fresh, moist adventitial surface on to a strip of dry chipboard in preparation for gross staining. They were then fixed and stained by the method described by Holman et al. (1958). This method consists of fixation of the specimens by their immersion in neutralized 10% formalin for 24 hours. After this period specimens were rinsed briefly in 70% alcohol, then immersed in Herxheimer's solution for 15 minutes at room temperature. The specimens were then differentiated in 80% alcohol for 20 minutes and following this were washed in running tap water for 1 hour.

The plaques and fatty streaks were not readily discernable by gross examination of the unstained tissue since the fibrous plaques were often so small that the slight elevation of the intimal surface could not be detected.

Measurement of the percent of the total area involved with atherosclerotic lesions was carried out by using a method of tracing and planimetry similar to that described by Cranston et al. (1964). No attempt was made to distinguish between plaques and fatty streaks, both were considered as lesions of atherosclerosis and were taken into account when calculating the percent of the total area involved. Figures 1 to 4 show stained and unstained aortas showing mildly (Figures 1 and 2) and severely (Figures 3 and 4) involved tissues.

The data collected from each experiment were statistically analyzed by variance methods described by Snedecor (1956).

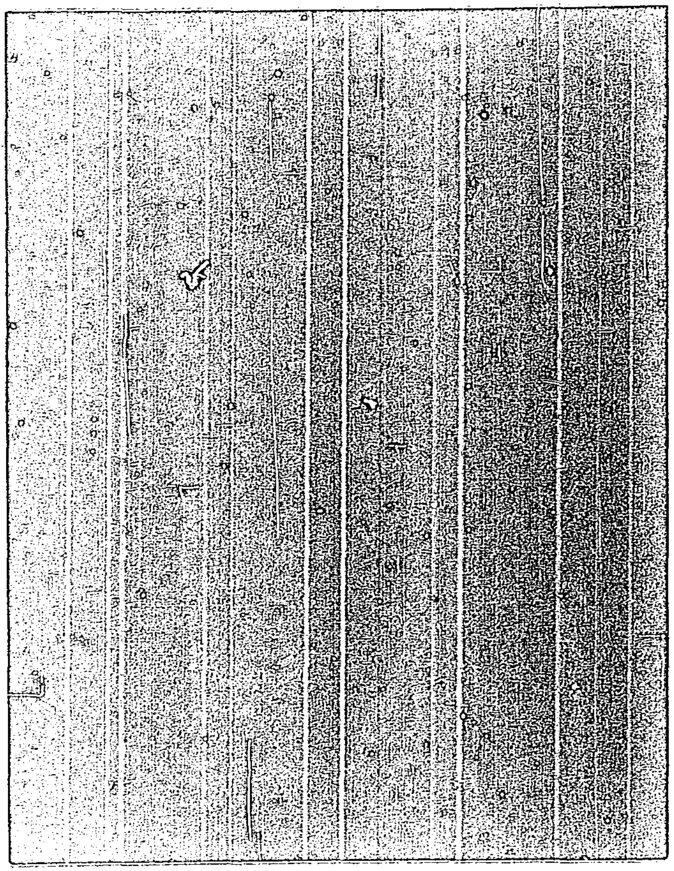


Figure 1. Unstained thoracic and abdominal aortas. Atherosclerotic lesions are not readily distinguishable. The dark areas are the result of blood staining.

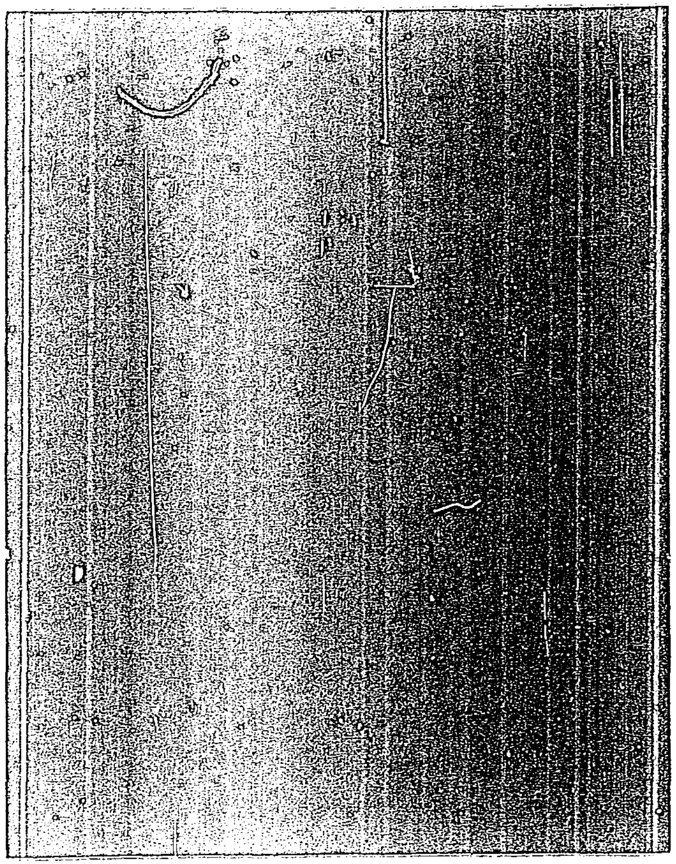


Figure 2. Thoracic and abdominal aortas as shown in Figure 1 after gross staining with Sudan IV. Atherosclerotic lesions are not readily distinguishable.

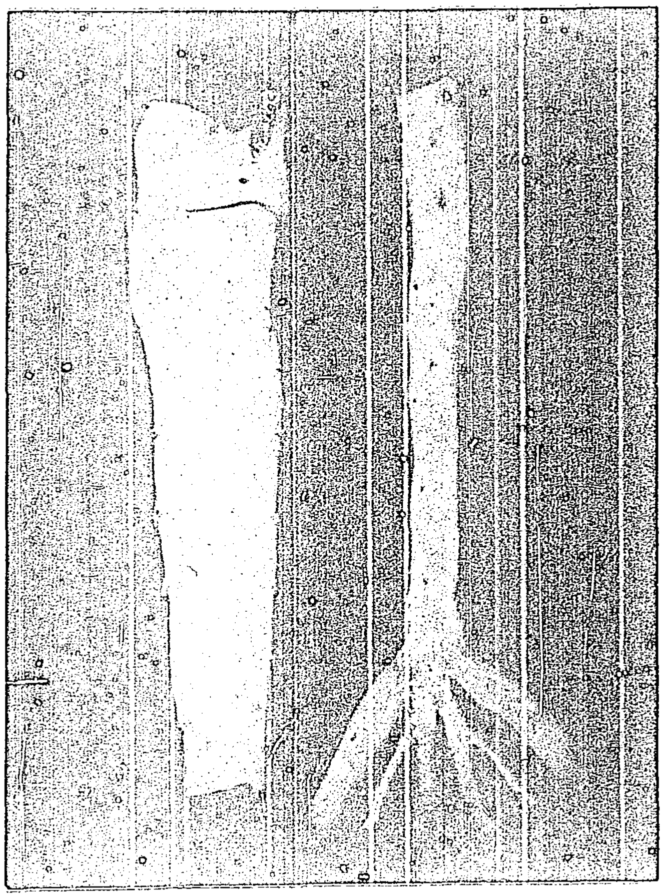


Figure 3. Unstained thoracic and abdominal aortas. Atherosclerotic lesions are not readily distinguishable.

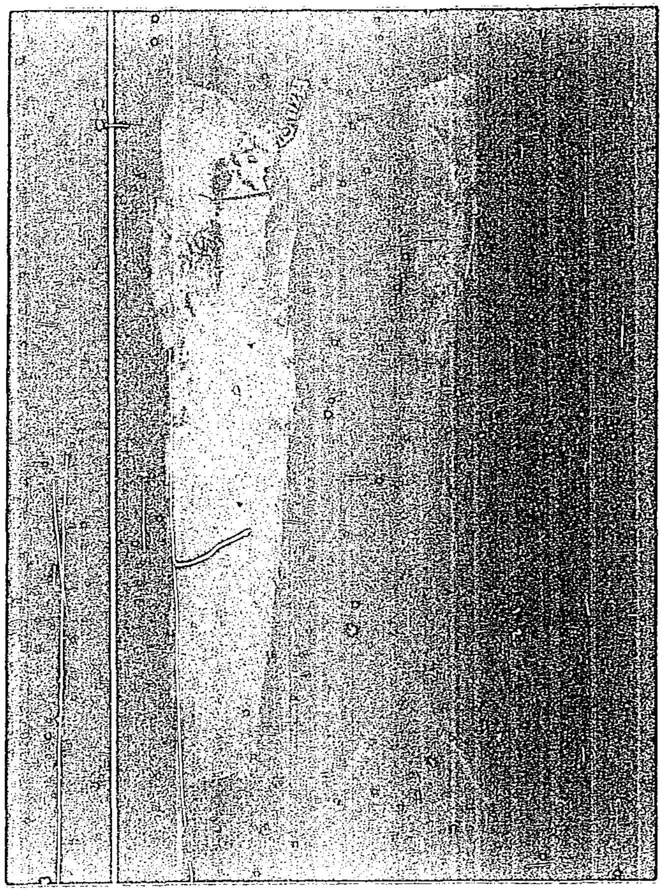


Figure 4. Thoracic and abdominal aortas as shown in Figure 3 after gross staining with Sudan IV. Atherosclerotic plaques and fatty streaks are readily distinguishable.

Experiment 6415 - Effect of Fat Source and Energy Intake on Serum Cholesterol and Lesion Formation

Objective

The purpose of this experiment was to study the effect of level of intake and source of fat (tallow versus soyoil) on levels of serum cholesterol and atherosclerotic lesions in the aorta and coronary arteries. A standard corn-soybean ration (Table 24) without added fat was also fed as a control.

Procedure

This experiment was conducted between April and September, 1964. Forty-eight pigs (8 pigs per treatment and 6 diet treatments) were randomly allotted to the diet treatments from outcome groups of weight within sex. The pigs were individually fed twice daily. One group of pigs was allowed to consume all of the control (low-fat) diet they wanted in the two 30-minute feeding periods daily. This level of intake was considered the high level of feed intake and served as the basis for determining the level of caloric intake for the other groups. A second group was fed 70% of the high level. The diets containing soyoil or tallow were then fed in isocaloric amounts to the high or low levels of intake. This resulted in a 2 X 3 factorial arrangement of two levels of feeding (high caloric or low caloric intake) in combination with three types of diets (low-fat control diet, diet containing tallow and diet containing soyoil).

Diets of composition shown in Appendix Tables 24, 25, 30 and 31 were fed throughout the trial. The pigs initially averaged 32.7 kg. bodyweight and 85 days of age.

Results and discussion

Summaries of serum cholesterol, food intake and atherosclerotic lesions in right coronary artery, left coronary artery, thoracic aorta and abdominal aorta are presented in Figures 5 to 9 and Tables 5 to 10. The analysis of variance plans and observed mean squares are presented in Appendix Tables 32 to 35.

The effect of fat source on serum cholestreol levels was significant ($P < .01$). Feeding the control diet resulted in significantly ($P < .01$) lower serum cholesterol levels than did the diets with added fat. Pigs consuming the tallow diet exhibited significantly ($P < .01$) higher serum cholesterol levels than did those consuming the soyoil diet. A significant fat source and level of feeding interaction ($P < .05$) was observed. The low intake on the tallow diet resulted in a higher serum cholesterol level than did the high intake level, the serum cholesterol levels for the two intake levels of the soyoil diet were about equal and the high intake level on the control diet resulted in a higher serum cholesterol level than did the low intake level.

The differences in serum cholesterol levels among samples taken at different times during the course of the experiment were statistically significant ($P < .01$). However, no consistent relationship of time on experiment and serum cholesterol level was readily apparent.

No statistical significance was noted in the analysis of the treatment effects on the percent of the total area of the right coronary artery affected with lesions. There was a trend, however, for the animals on the lower levels of intake to have a lesser area affected with lesions.

Figure 5. Experiment 6415 - Effect of fat source and level of intake on serum cholesterol levels.

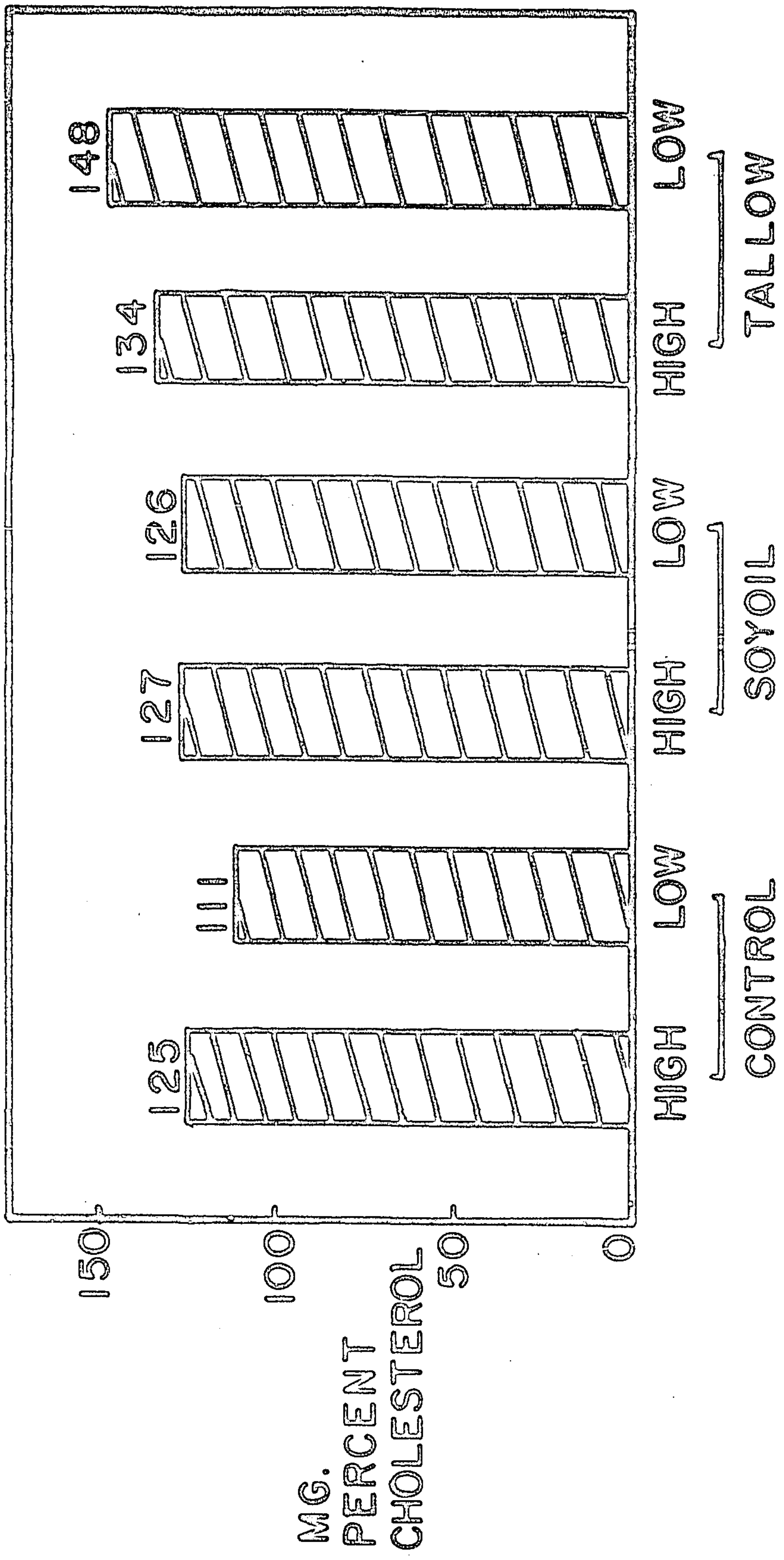

MG.
PERCENT
CHOLESTEROL
150
100
50
0
125
111
127
126
134
148
HIGH
LOW
HIGH
LOW
HIGH
LOW
CONTROL
SOYOIL
TALLOW

Figure 6. Experiment 6415 - Effect of fat source and level of intake on percent of the total area of the right coronary artery affected by lesions.

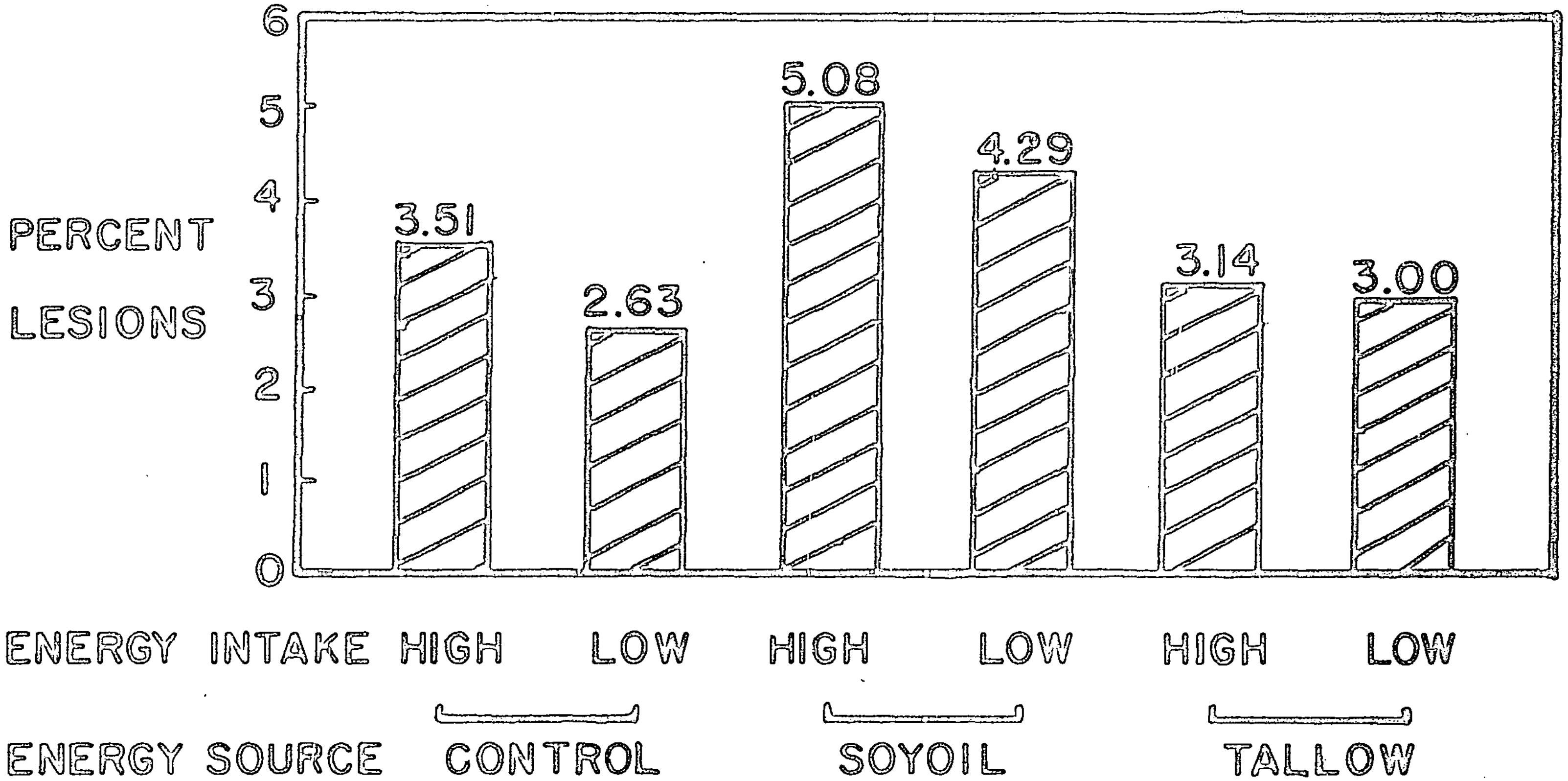

6
5
4
3
2
1
0
PERCENT
LESIONS
3.51
2.63
5.08
4.29
3.14
3.00
ENERGY INTAKE HIGH LOW HIGH LOW HIGH LOW
ENERGY SOURCE CONTROL SOYOIL TALLOW

Figure 7. Experiment 6415 - Effect of fat source and level of intake on percent of the total area of the left coronary artery affected by lesions.

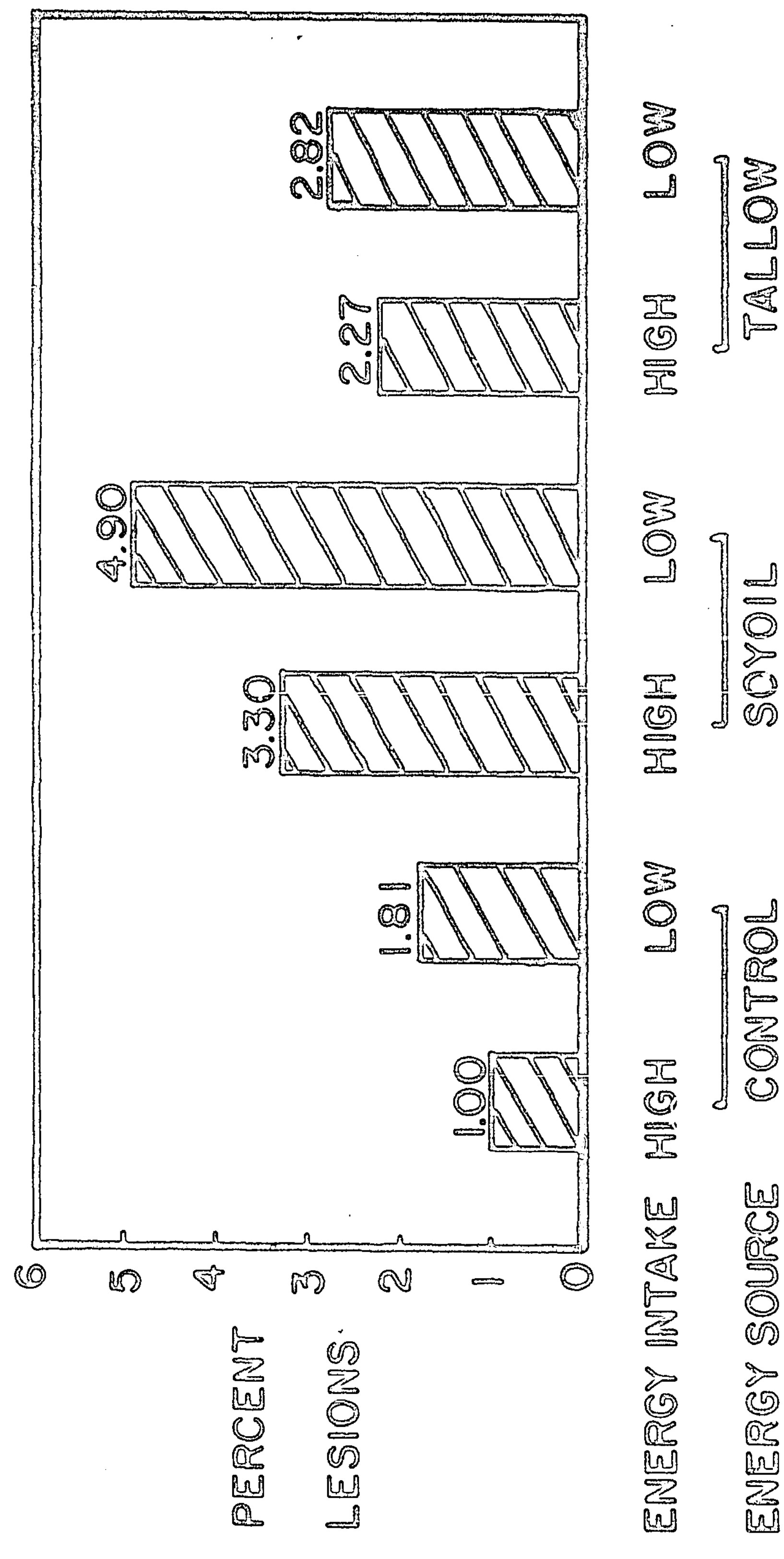
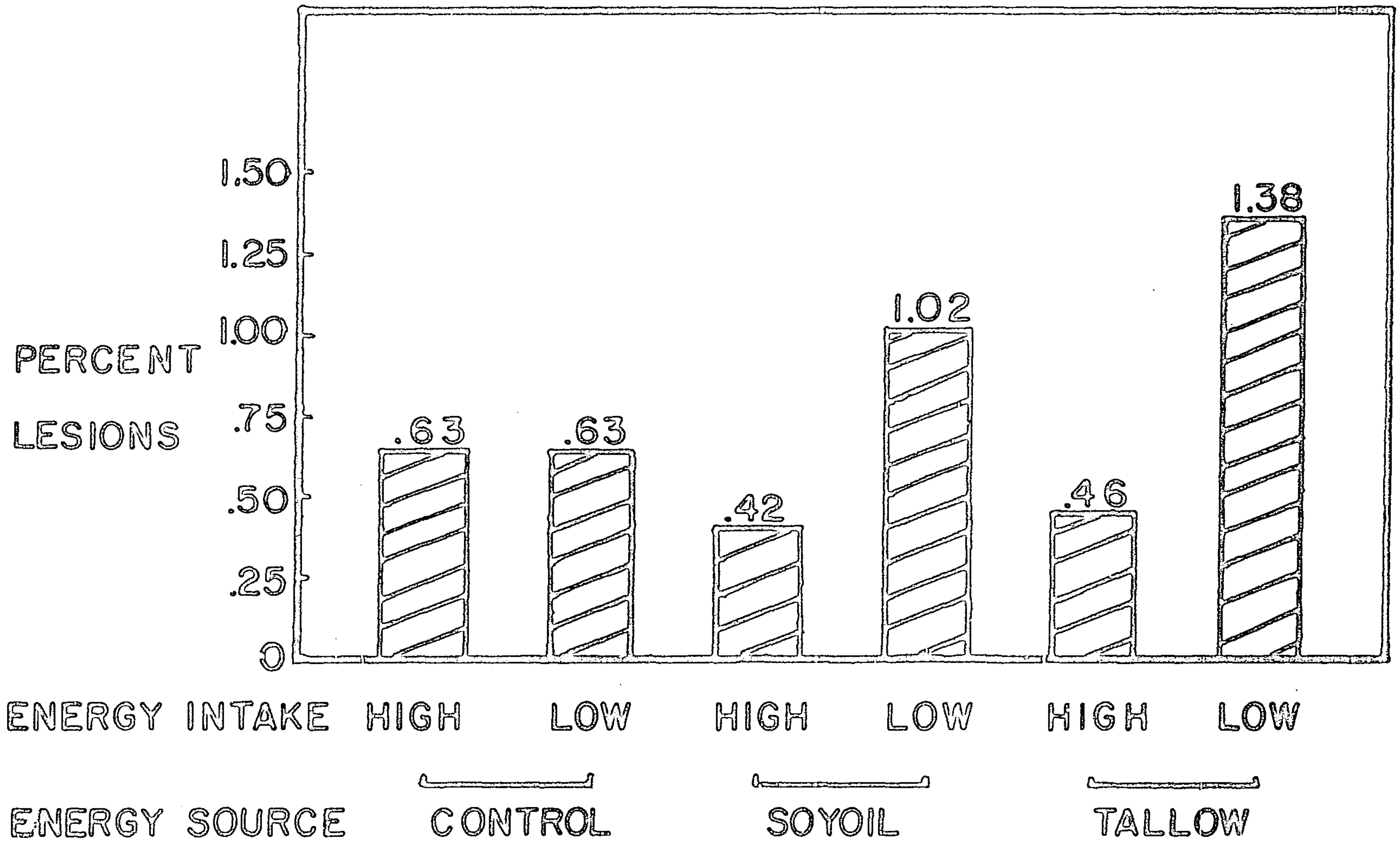
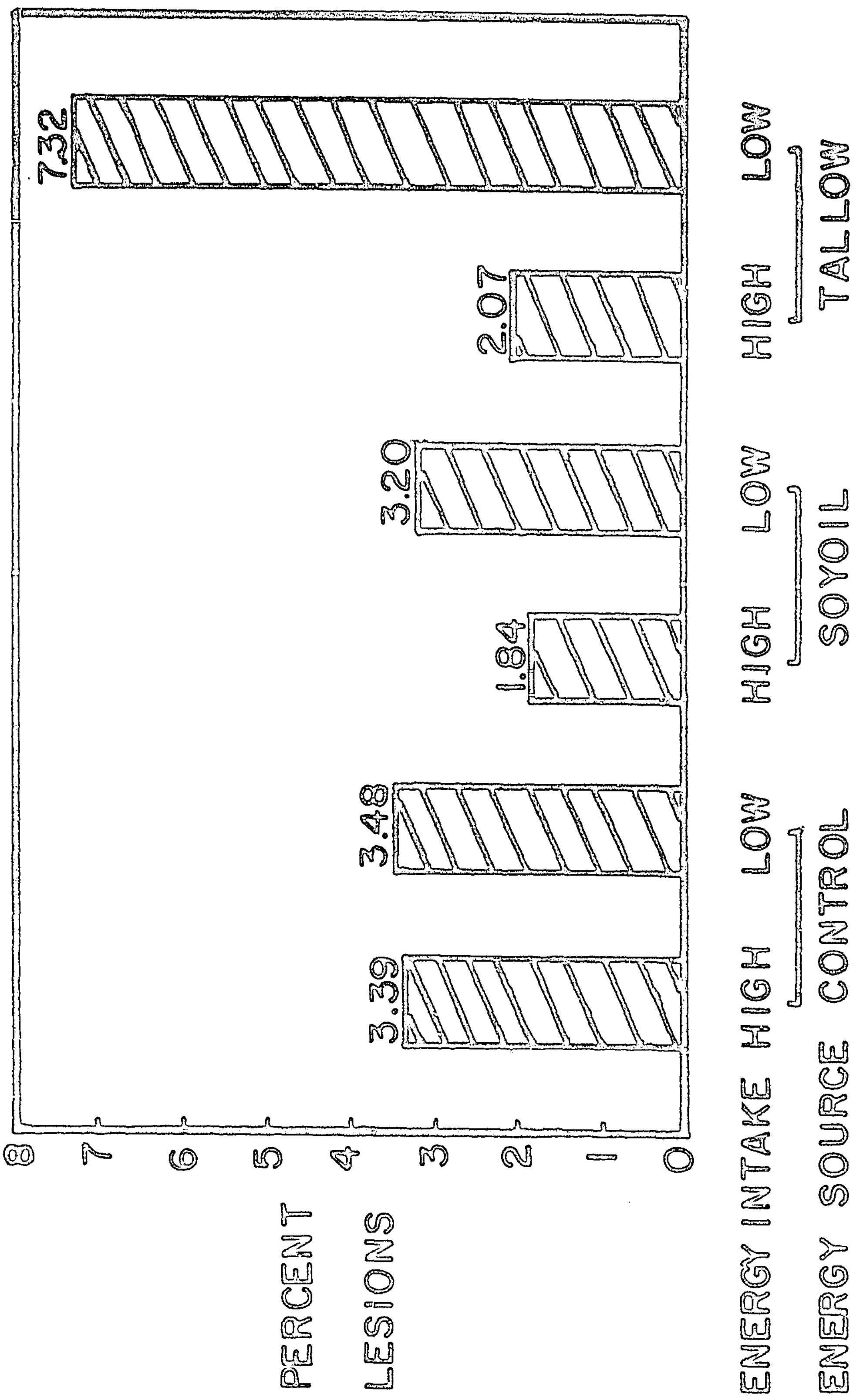

PERCENT LESIONS
0
1
2
3
4
5
6
1.00
1.81
3.30
4.90
2.27
2.82
ENERGY INTAKE
HIGH
LOW
HIGH
LOW
HIGH
LOW
ENERGY SOURCE
CONTROL
SOYOIL
TALLOW

Figure 8. Experiment 6415 - Effect of fat source and level of intake on percent of the total area of the thoracic aorta affected by lesions.



PERCENT
LESIONS
1.50
1.25
1.00
.75
.50
.25
0
.63
.63
.42
1.02
.46
1.38
ENERGY INTAKE
HIGH
LOW
HIGH
LOW
HIGH
LOW
ENERGY SOURCE
CONTROL
SOYOIL
TALLOW

Figure 9. Experiment 6415 - Effect of fat source and level of intake on percent of the total area of the abdominal aorta affected by lesions.


PERCENT
LESIONS
0
1
2
3
4
5
6
7
8
3.39
3.48
1.84
3.20
2.07
732
ENERGY INTAKE HIGH LOW HIGH LOW HIGH LOW
ENERGY SOURCE CONTROL SOYOIL TALLOW

Pigs fed soyoil had the greatest incidence of lesions, those fed tallow and the control diet being equal. Statistical analysis of the effect of the treatments on the percent of the total area of the left coronary artery affected by lesions showed that the addition of fat to the diet resulted in a significantly higher ($P < .05$) incidence of lesions. Fat source did not significantly affect the production of lesions; however, the following trend was noted in decreasing order of severity: soyoil, tallow and control diets. The pigs had a greater incidence of lesions in the right coronary artery than in the left coronary artery.

No statistically significant effects of dietary variables were noted in the production of atherosclerotic lesions in the thoracic aorta. The following trend was noted in decreasing order of severity of lesion production: tallow, soyoil and control diets. The animals on the low intake tended to have a greater percent of lesions than those on the high dietary intake level. In the abdominal aorta, pigs on the high energy intake level exhibited a significantly ($P < .05$) lower percent of lesions than did those on the low energy intake. Fat source exhibited no significant effects; however, in order of decreasing severity of lesion involvement, the treatments were: tallow, control and soyoil. The pigs had a greater incidence of lesions in the abdominal aorta than in the thoracic aorta.

Experiment 6434 - Effect of Fat Source, Energy Intake and Protein Level on Serum Cholesterol and Lesion Formation

Objective

The purpose of this experiment was to study the effect of level of energy intake, level of protein (18% versus 12%) and source of fat

Table 5. Experiment 6415 - Summary of the effect of fat source and level of intake on serum cholesterol levels (mg. percent)

	Treatment					
Fat source	Control		Soyoil		Tallow	
Level of intake	High	Low	High	Low	High	Low
Time on experiment (wks.)						
0	134[a]	110	111	117	118	120
2	119	116	135	130	143	155
4	178	160	198	196	209	212
6	136	124	138	143	157	170
8	101	98	110	114	118	128
10	136	114	123	134	128	159
12	134	120	142	131	142	152
14	127	115	122	124	131	160
16	115[b]	93	107	115	119	129
18	96[c]	93	115	102	100	122
20	98[c]	81	100	84	104	123
Average	125	111	127	126	134	148

[a]All values represent the average for eight pigs unless noted otherwise.

[b]Each value for 16 weeks on experiment represents the average for seven pigs.

[c]Each value for 18 and 20 weeks on experiment represents the average for four pigs.

Table 6. Experiment 6415 - Summary of average daily food intake (Kg.)

Fat source		Treatment					
		Control		Soyoil		Tallow	
Level of intake		High	Low	High	Low	High	Low
Replication	Block						
1	A	2.44	1.59	1.89	1.36	1.86	1.33
	B	2.51	1.67	1.98	1.39	1.95	1.37
2	A	2.32	1.55	1.82	1.32	1.79	1.28
	B	2.49	1.65	1.95	1.40	2.11	1.36
3	A	2.36	1.58	1.88	1.34	1.85	1.31
	B	2.48	1.67	2.00	1.42	1.93	1.38
4	A	2.40	1.58	1.88	1.33	1.84	1.32
	B	2.52	1.66	2.00	1.41	1.93	1.39
Average		2.44	1.62	1.92	1.37	1.91	1.34
Energy Intake[a]		7552	5014	7037	5021	7147	5014
Percent cal. from added fat		0	0	29.8	29.8	31.2	31.2

[a]Kilocalories of metabolizable energy consumed daily .

Table 7. Experiment 6415 - Summary of the effect of fat source and level of intake on percent lesions in right coronary artery

Fat source		Treatment					
		Control		Soyoil		Tallow	
Level of intake		High	Low	High	Low	High	Low
Replication	Block						
1	A	--	--	--	--	--	--
	B	0.00[a]	0.00	12.24	9.46	3.68	0.00
2	A	10.64	6.67	6.45	0.00	4.60	6.76
	B	3.85	3.12	3.16	3.61	6.06	0.00
3	A	5.26	3.19	3.30	1.30	0.00	0.00
	B	4.04	0.00	9.09	10.00	0.00	4.03
4	A	0.00	4.28	1.32	5.66	7.63	0.00
	B	0.80	1.15	0.00	0.00	0.00	10.00
Average		3.51	2.63	5.08	4.29	3.14	3.00

[a] Each value represents the percent of the total area of the right coronary artery affected by atherosclerotic lesions.

Table 8. Experiment 6415 - Summary of the effect of fat source and level of intake on percent lesions in left coronary artery

Fat source / Level of intake		Treatment: Control High	Control Low	Soyoil High	Soyoil Low	Tallow High	Tallow Low
Replication	Block						
1	A	0.00[a]	0.00	3.45	5.88	1.78	1.92
	B	0.00	0.00	2.24	10.00	0.00	0.00
2	A	6.25	10.00	5.75	5.63	7.02	17.46
	B	0.00	0.00	0.00	0.00	0.00	0.00
3	A	0.00	0.00	0.93	0.00	5.21	0.00
	B	1.74	0.00	8.47	12.26	0.00	0.00
4	A	0.00	2.13	5.56	5.47	4.17	3.20
	B	0.00	2.36	0.00	0.00	0.00	0.00
Average		1.00	1.81	3.30	4.90	2.27	2.82

[a]Each value represents the percent of the total area of the left coronary artery affected by atherosclerotic lesions.

Table 9. Experiment 6415 - Summary of the effect of fat source and level of intake on percent lesions in thoracic aorta

Fat source		Treatment					
		Control		Soyoil		Tallow	
Level of intake		High	Low	High	Low	High	Low
Replication	Block						
1	A	0.86[a]	0.88	0.33	4.15	0.51	6.97
	B	0.00	0.74	0.58	0.00	0.60	0.00
2	A	0.47	0.80	0.67	0.64	0.38	2.45
	B	0.00	0.00	0.81	1.39	0.00	0.09
3	A	1.19	0.00	0.47	1.36	0.31	0.54
	B	1.75	0.40	0.00	0.00	0.00	0.00
4	A	0.80	0.75	0.46	0.00	1.21	1.00
	B	0.00	1.50	0.00	0.66	0.66	0.00
Average		0.63	0.63	0.42	1.02	0.46	1.38

[a]Each value represents the percent of the total area of the thoracic aorta affected by atherosclerotic lesions.

Table 10. Experiment 6415 - Summary of the effect of fat source and level of intake on percent lesions in abdominal aorta

Fat source		Treatment					
		Control		Soyoil		Tallow	
Level of intake		High	Low	High	Low	High	Low
Replication	Block						
1	A	1.47[a]	4.75	2.57	2.13	3.06	9.80
	B	0.00	3.69	1.35	0.56	0.00	12.96
2	A	6.56	8.35	1.05	0.75	4.59[b]	15.15
	B	3.04	1.36	1.58	7.01	3.14	6.70
3	A	1.61	1.10	0.00	7.29	0.50	4.21
	B	7.69	7.10	3.74	1.46	1.60	0.30
4	A	3.13	0.00	2.14	3.83	3.68	2.90
	B	3.64	1.53	2.31	2.61	0.00	6.54[b]
Average		3.39	3.48	1.84	3.20	2.07	7.32

[a]Each value represents the percent of the total area of the abdominal aorta affected by atherosclerotic lesions.

[b]Calculated missing value.

(tallow versus soyoil) on levels of serum cholesterol and atherosclerotic lesions in the aorta and cardiac arteries. The diets were diluted with ground corn cobs to increase the proportion of total calories coming from the fat source. Corn cobs are highly indigestible, thus contributing little energy to the diet and in addition are highly absorptive which permits the including of higher levels of fat in the diet mixture.

Throughout the previous experiment, blood samples were taken from the pigs following a 16-hour fast. It is possible that treatment effects may be less evident in the fasted than in the non-fasted animals. To determine if the degree of fasting altered the treatment effects, samples were collected prior to feeding after a 16-hour fast and at 2, 4 and 6 hours after feeding. These collections were made as follows: all pigs were bled after a 16-hour fast and then fed. After a period of 2 hours two replications of the pigs were sampled, after 4 hours two more replications were sampled, and after 6 hours the remaining two replications were sampled. This method was adopted since the facilities did not permit and also it would have been extremely hard on the pigs, which had been on experiment for a long time and subjected to periodic bleeding, to withdraw samples from each pig at the 2-hour intervals.

Procedure

The experiment was conducted between October, 1964 and June, 1965. Forty-eight pigs (6 pigs per treatment and 8 diet treatments) were randomly allotted to diet treatments from outcome groups of weight within sex. The pigs were individually fed twice daily. The pigs on the high level of feed intake were allowed approximately full feed, and this was increased stepwise up to a maximum of 2.7 kg. as the pigs matured. The pigs were purposely restricted to slightly below full-feed intake to insure that each pig consumed the amount of food provided. The second group was fed 70% of the high level of intake. This resulted in a 2 x 2 x 2 factorial arrangement of two levels of feeding (high caloric or low caloric intake), two types of diet (diet containing soyoil and diet containing tallow) and two levels of protein (12% and 18%).

Diets of composition as shown in Appendix Tables 26, 27, 30 and 31 were fed throughout the trial. The average initial weight of the pigs was 34.7 kg. and the average age was 101 days.

Results and discussion

Summaries of serum cholesterol, food intake and atherosclerotic lesions in right coronary artery, left coronary artery, thoracic aorta and abdominal aorta are presented in Figures 10 to 15 and Tables 11 to 17. The analysis of variance plans and observed mean squares are presented in Appendix Tables 36 to 39.

Fat source had no statistically significant effect on serum cholesterol levels. A trend however was present, in that the tallow treatment resulted in higher serum cholesterol levels than did the soyoil. Overall, the low intake groups had somewhat similar serum cholesterol values to those of the high intake groups. The group receiving the diets containing 12% protein tended to have higher serum cholesterol levels than did the group receiving the diets containing 18% protein. Time after feeding resulted in a significant ($P < .05$) increase in serum cholesterol levels, with the greatest increase generally coming between the 4 and 6 hour sampling periods. The group receiving the high intake level of the diet containing soyoil and 12% protein, however did not show this and instead, with this treatment, the serum cholesterol value dropped considerably between 4 and 6 hours. In this effect of time after feeding on serum cholesterol levels, the feeding of soyoil diets resulted in significantly ($P < .05$) lower levels than did the feeding of the tallow diets.

The feeding of tallow diets resulted in a trend toward an increased incidence of lesions in the right and left coronary arteries, and pigs fed the 18% protein diets exhibited a trend toward an increased incidence of lesions over those fed the 12% protein diets. In the case of the left coronary artery there was a significant protein level, fat source and level of feeding interaction ($P < .05$); pigs on the high intake of the high protein diet containing tallow having the greater incidence of lesions. The high level of intake resulted in a greater incidence of lesions than did the low intake level in the right and left coronary arteries; however, the difference was not statistically significant. The pigs had a greater incidence of lesions in the left coronary artery than in the right coronary artery.

There were no statistically significant treatment effects on the incidence of lesions in the thoracic or abdominal aortas. The pigs had a greater incidence of lesions in the abdominal aorta than in the thoracic aorta. The feeding of low protein, low intake and tallow diets resulted in a greater incidence of lesions in the thoracic aortas than did the feeding of high protein, high intake and soyoil diets; however, the differences were not statistically significant. Differences similar to the above were noted with the abdominal aorta, with the exception that the high intake level in this case resulted in a greater incidence of lesions than did the low intake level. A probable explanation for this reversal was an extremely high individual value observed in the group receiving the high intake level of the diet containing tallow and 12% protein.

Experiment 6512 - Effect of Cholesterol Addition to Different Fat Sources on Serum Cholesterol and Lesion Formation

Objective

The purpose of this experiment was to study the effect of the addition of 1% cholesterol to diets containing 14% protein and either 5% soy-oil or 5% tallow, on levels of serum cholesterol and atherosclerotic lesions in the aorta and cardiac arteries.

Procedure

This experiment was conducted during the months of April to July, 1965. Forty-eight pigs were randomly allotted to 4 diet treatments (3 pigs per pen and 4 pens per diet treatment) from outcome groups based on initial weight and sex. The pigs were offered feed *ad libitum*.

Diets of composition as shown in Appendix Tables 28 to 32 were fed throughout the trial. The average initial weight of the pigs was 35 kg. and the average age was 98 days.

Results and discussion

Summaries of serum cholesterol, food intake and atherosclerotic lesions in right coronary artery, left coronary artery, thoracic aorta

Figure 10. Experiment 6434 - Effect of fat source, protein level and level of intake on serum cholesterol levels.

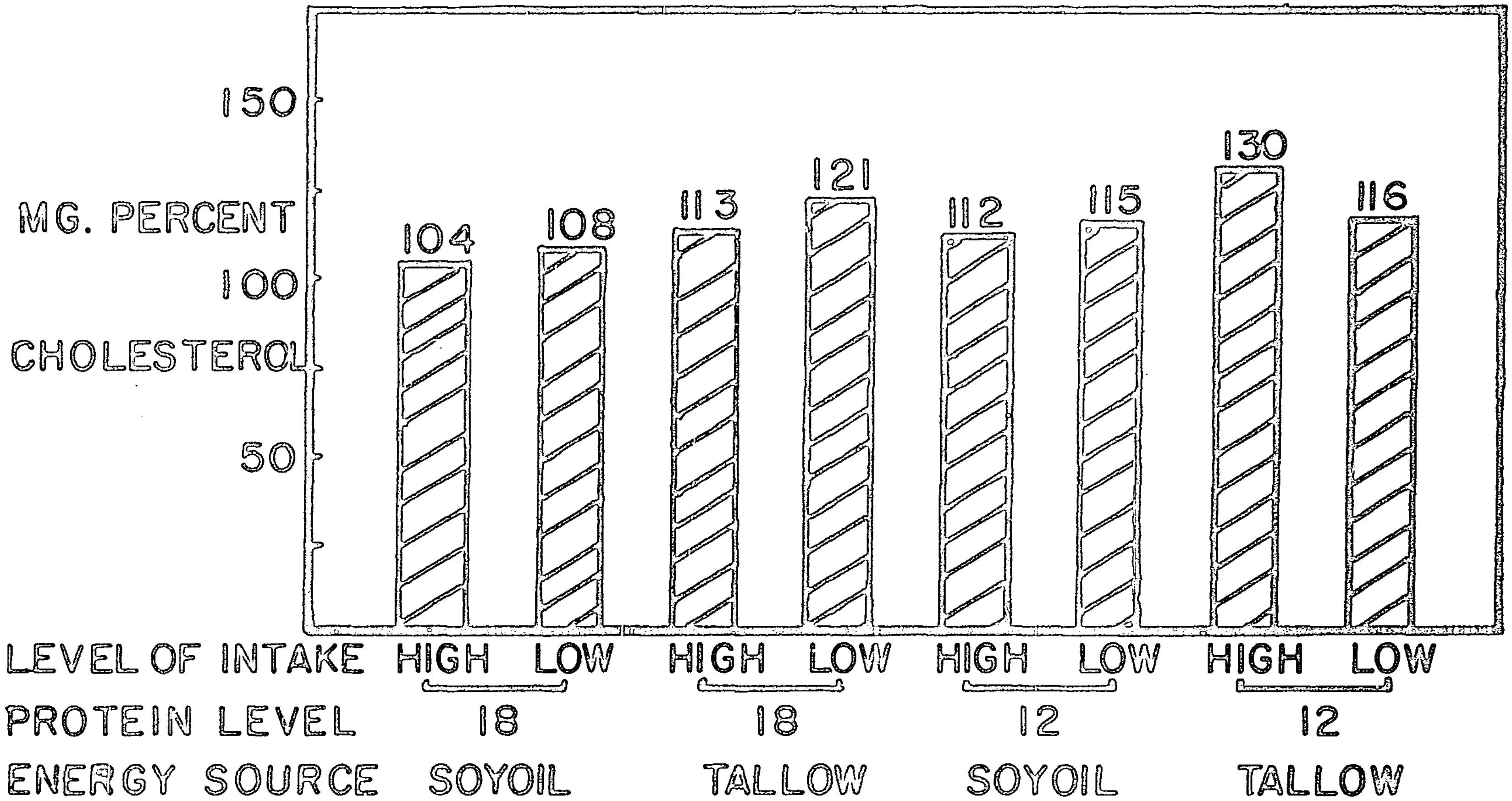

150
MG. PERCENT
100
CHOLESTEROL
50
104
108
113
121
112
115
130
116
LEVEL OF INTAKE HIGH LOW HIGH LOW HIGH LOW HIGH LOW
PROTEIN LEVEL 18 18 12 12
ENERGY SOURCE SOYOIL TALLOW SOYOIL TALLOW

Figure 11. Experiment 6434 - Effect of time after feeding on serum cholesterol levels

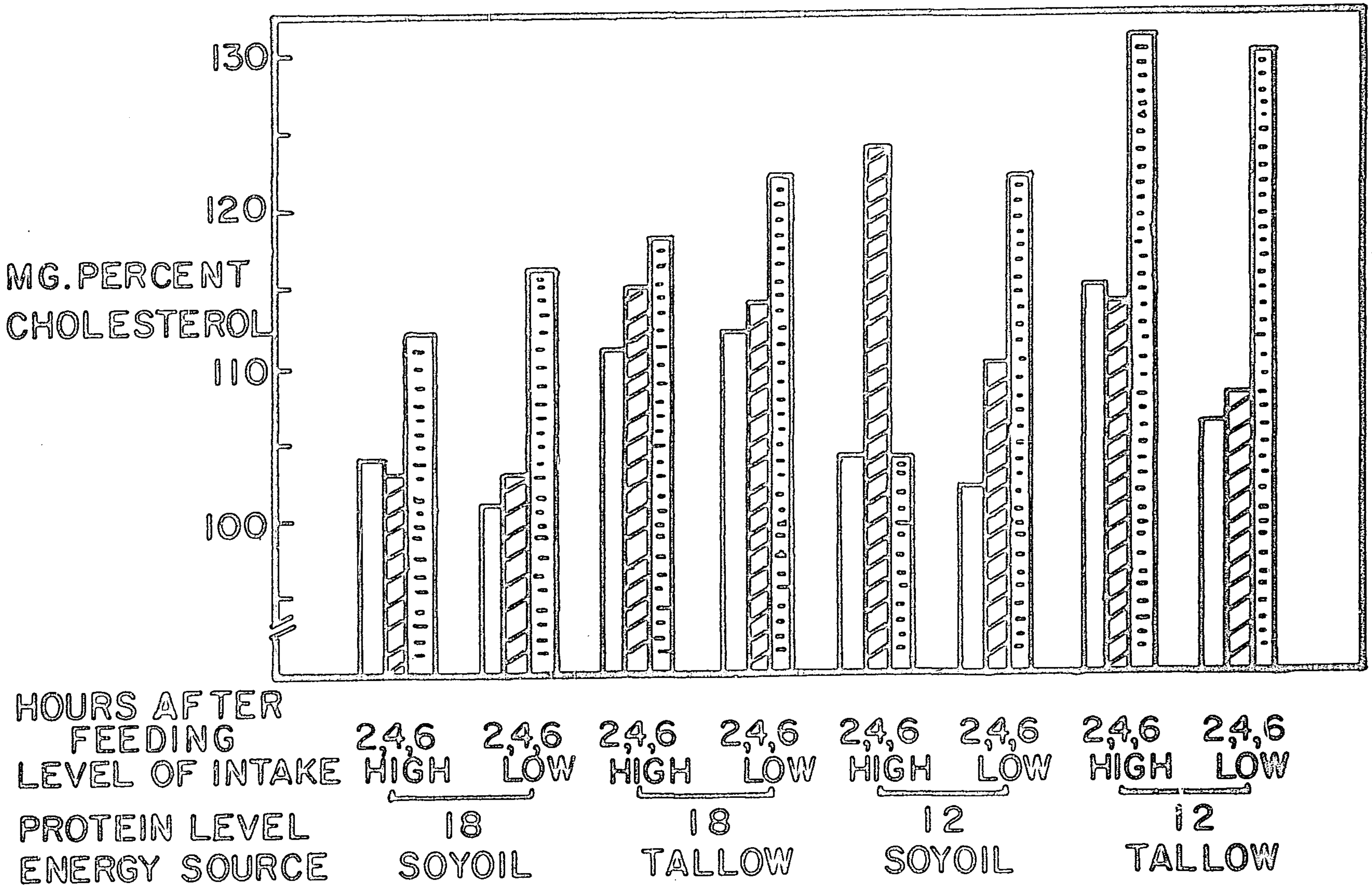

MG. PERCENT CHOLESTEROL
130
120
110
100
HOURS AFTER FEEDING
2,4,6 2,4,6 2,4,6 2,4,6 2,4,6 2,4,6 2,4,6 2,4,6
LEVEL OF INTAKE HIGH LOW HIGH LOW HIGH LOW HIGH LOW
PROTEIN LEVEL 18 18 12 12
ENERGY SOURCE SOYOIL TALLOW SOYOIL TALLOW

Figure 12. Experiment 6434 - Effect of fat source, protein level and level of intake on percent of the total area of the right coronary artery affected by lesions.

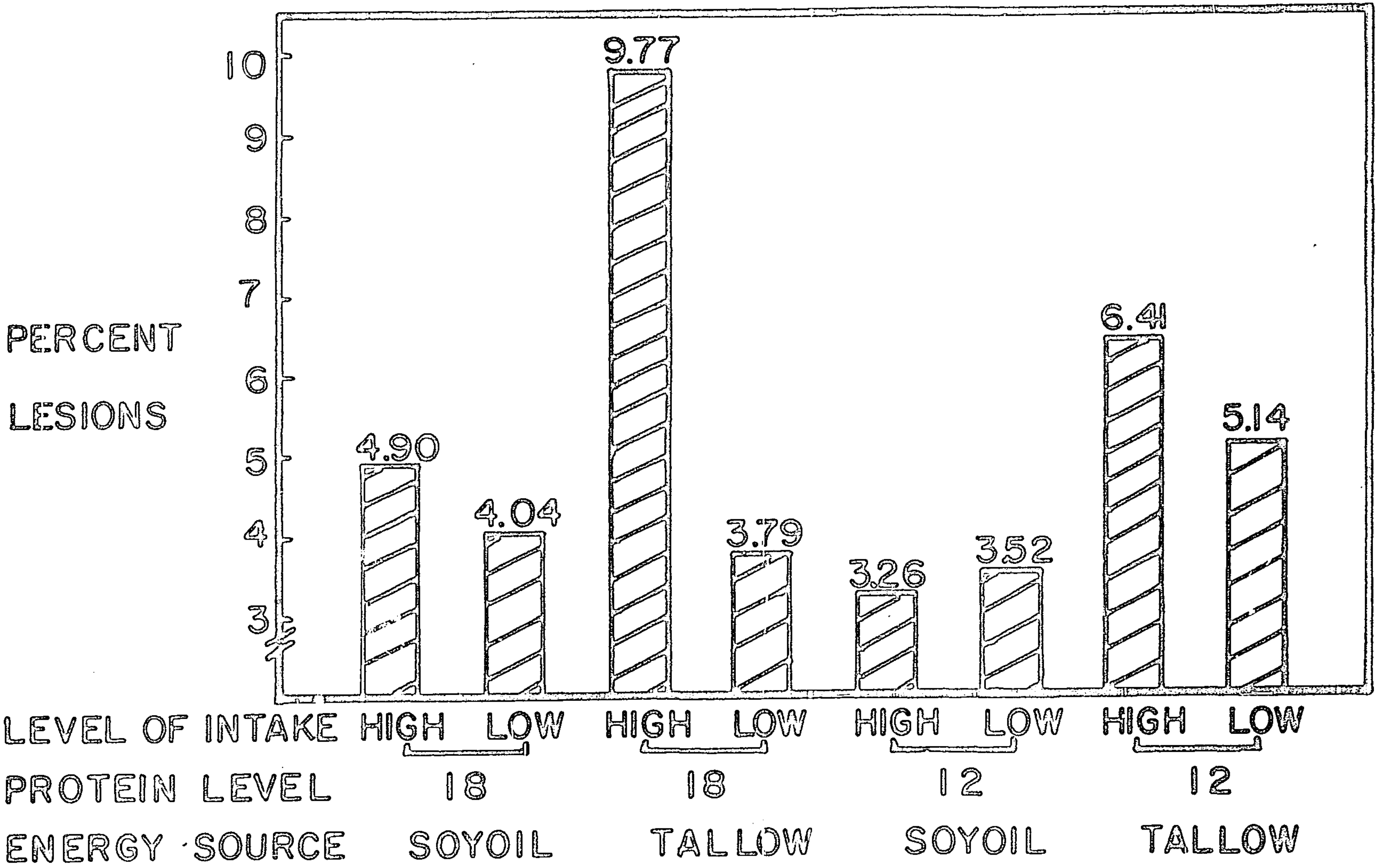

PERCENT LESIONS
10
9
8
7
6
5
4
3
9.77
6.41
5.14
4.90
4.04
3.79
3.26
3.52
LEVEL OF INTAKE HIGH LOW HIGH LOW HIGH LOW HIGH LOW
PROTEIN LEVEL 18 18 12 12
ENERGY SOURCE SOYOIL TALLOW SOYOIL TALLOW

Figure 13. Experiment 6434 - Effect of fat source, protein level and level of intake on percent of the total area of the left coronary artery affected by lesions.

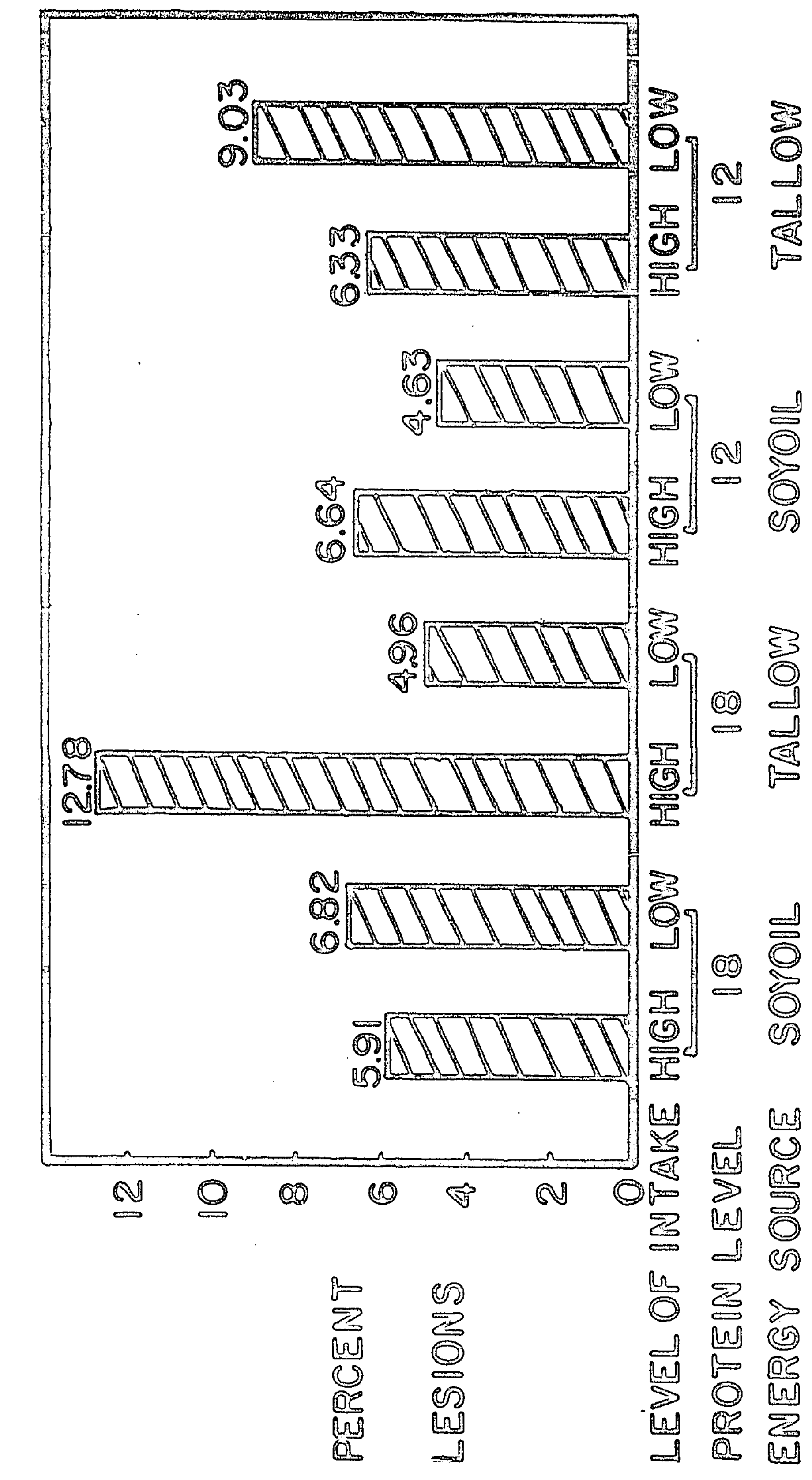
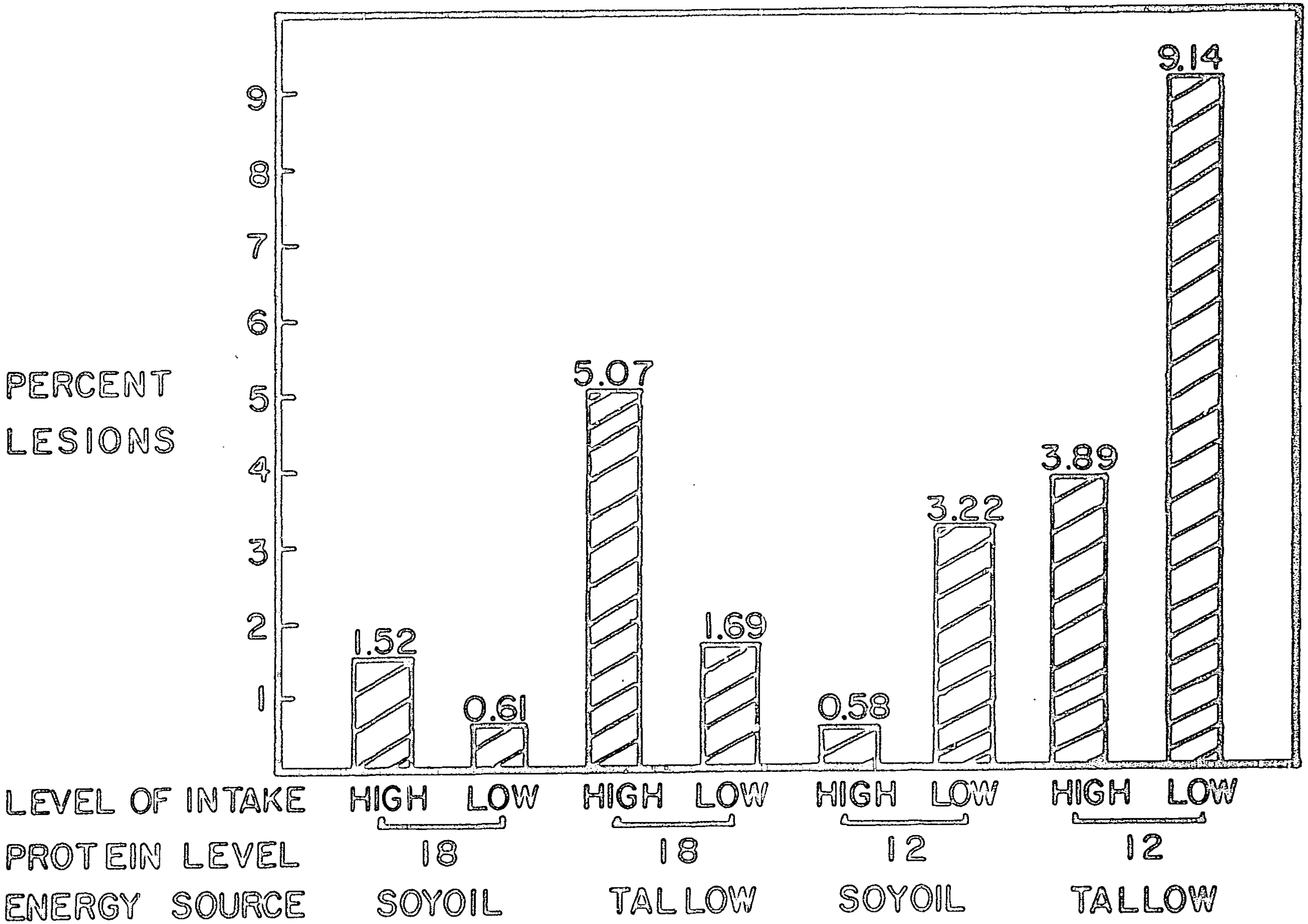

PERCENT LESIONS
0
2
4
6
8
10
12
5.91
6.82
12.78
4.96
6.64
4.63
6.33
9.03
LEVEL OF INTAKE
HIGH LOW HIGH LOW HIGH LOW HIGH LOW
PROTEIN LEVEL
18 18 12 12
ENERGY SOURCE
SOYOIL TALLOW SOYOIL TALLOW

Figure 14. Experiment 6434 - Effect of fat source, protein level and level of intake on percent of the total area of the thoracic aorta affected by lesions.



PERCENT
LESIONS
9
8
7
6
5
4
3
2
1
1.52
0.61
5.07
1.69
0.58
3.22
3.89
9.14
LEVEL OF INTAKE
HIGH
LOW
HIGH
LOW
HIGH
LOW
HIGH
LOW
PROTEIN LEVEL
18
18
12
12
ENERGY SOURCE
SOYOIL
TALLOW
SOYOIL
TALLOW

Figure 15. Experiment 6434 - Effect of fat source, protein level and level of intake on percent of the total area of the abdominal aorta affected by lesions.

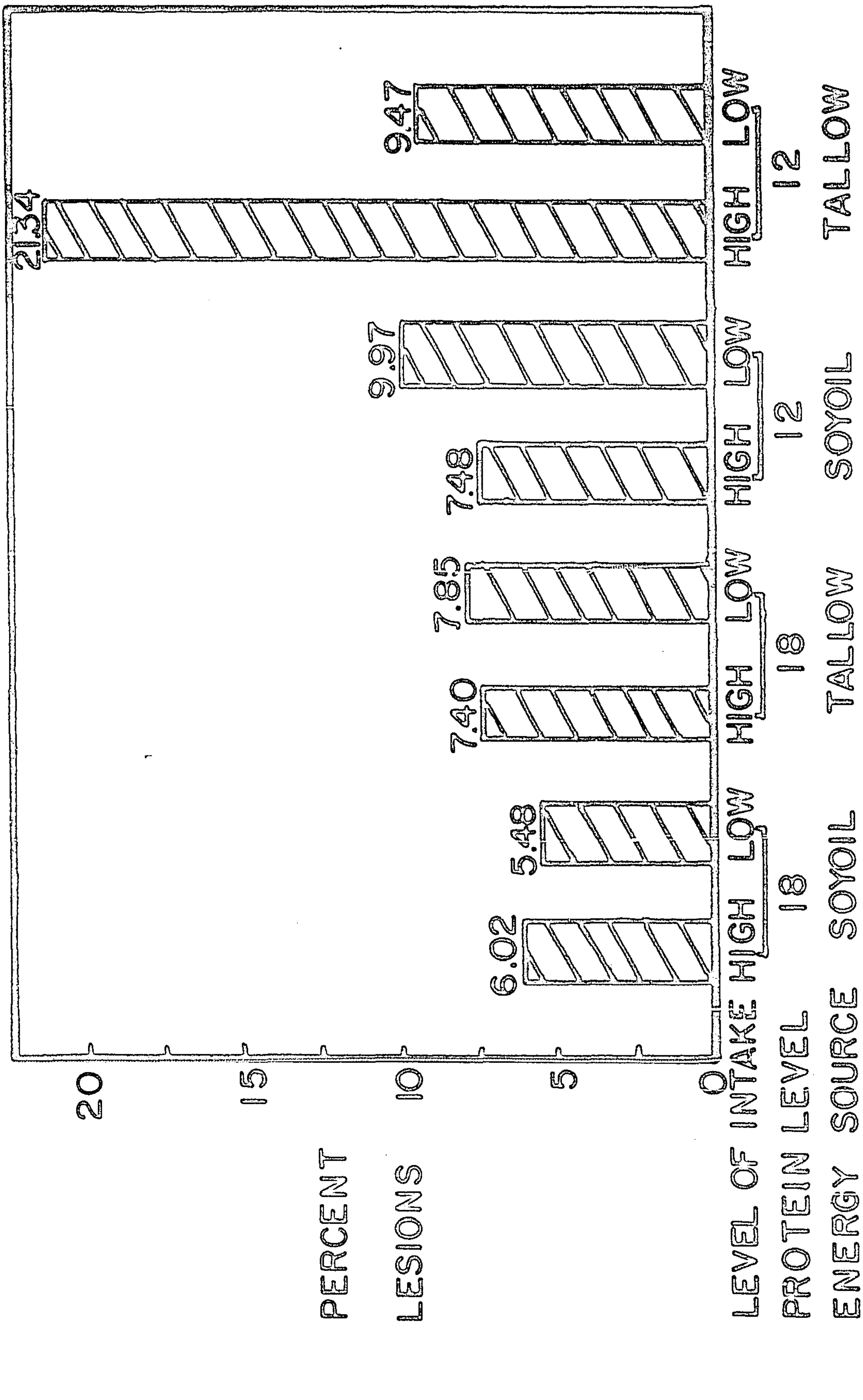

PERCENT LESIONS
0
5
10
15
20
6.02
5.48
7.40
7.85
7.48
9.97
21.34
9.47
LEVEL OF INTAKE HIGH LOW HIGH LOW HIGH LOW HIGH LOW
PROTEIN LEVEL 18 18 12 12
ENERGY SOURCE SOYOIL TALLOW SOYOIL TALLOW

Table 11. Experiment 6434 - Summary of the effect of fat source, protein level and level of intake on serum cholesterol levels (mg. percent)

Protein level, %	18				12			
Fat source	Soyoil		Tallow		Soyoil		Tallow	
Intake level	High	Low	High	Low	High	Low	High	Low
Time on experiment (wks.)								
0	98[a]	106	93	114	98	98	117	107
2	101	105	107	131	103	108	138	120
4	100	106	108	121	125	136	137	131
6	112	118	113	137	114	111	134	122
8	102	108	117	118	122	126	139	114
10	119	121	114	139	117	121	139	117
12	112	117	127	139	133	128	142	118
14	110	112	110	129	115	113	139	123
16[b]	114	111	129	124	126	127	141	131
20	114	113	126	134	122	120	142	118
22	118	130	130	127	131	131	141	120
24	108	117	122	123	119	124	134	119
26	84	84	93	96	91	94	101	100
28	82	84	91	91	88	90	96	100
30	103	104	112	108	104	113	123	107
32	101	98	111	118	105	112	126	116
34	96	104	113	111	99	111	117	117
Average	104	108	113	121	112	115	130	116

[a]Each value represents the average for six pigs .

[b]Bleeding at 18 weeks on experiment was omitted because of quarantine measures in operation as a result of an outbreak of transmissible gastroenteritis .

Table 12. Experiment 6434 - Summary of the effect of time after feeding on serum cholesterol levels (mg. percent)

Treatment								
Protein level, %	18				12			
Fat source	Soyoil		Tallow		Soyoil		Tallow	
Intake level	High	Low	High	Low	High	Low	High	Low
Fasting level[a]	97[b]	97	109	107	98	103	114	103
2 hr. after feeding[c]	104	101	111	112	104	102	115	106
Fasting level	90	94	108	107	106	106	107	107
4 hr. after feeding	103	103	115	114	124	110	114	108
Fasting level	100	102	103	106	92	110	125	119
6 hr. after feeding	112	116	118	122	104	122	131	130

[a]Fasting serum cholesterol levels were obtained on all the animals.

[b]Each value represents the average of eight values.

[c]The hours after feeding levels were obtained from separate replications in each case.

Table 13. Experiment 6434 - Summary of average daily food intake (Kg.)

		Treatment							
Protein level, %		18				12			
Fat source		Soyoil		Tallow		Soyoil		Tallow	
Intake level		High	Low	High	Low	High	Low	High	Low
Replication	Block								
1	A	2.29	1.62	2.28	1.60	2.27	1.62	2.30	1.61
	B	2.31	1.63	2.30	1.63	2.28	1.63	2.31	1.63
2	A	2.28	1.61	2.25	1.59	2.28	1.62	2.24	1.60
	B	2.31	1.63	2.27	1.61	2.31	1.63	2.27	1.62
3	A	2.27	1.63	2.29	1.63	2.26	1.63	2.27	1.60
	B	2.29	1.64	2.30	1.64	2.27	1.63	2.28	1.61
Average		2.29	1.63	2.28	1.62	2.28	1.63	2.28	1.61
Energy intake[a]		6570	4676	6721	4776	6801	4862	6981	4930
Percent cal. from fat source		38.1		39.8		36.6		38.2	

[a]Kilocalories of metabolizable energy consumed per day.

Table 14. Experiment 6434 - Summary of the effect of fat source, protein level and level of intake on percent lesions in right coronary artery

		Treatment							
Protein level, %		18				12			
Fat source		Soyoil		Tallow		Soyoil		Tallow	
Intake level		High	Low	High	Low	High	Low	High	Low
Replication	Block								
1	A	5.74[a]	0.00	15.20	0.79	4.14	3.17	1.77	0.00
	B	5.77	1.43	7.75	3.28	2.40	0.00	0.00	0.62
2	A	1.19	4.10	1.24	0.92	3.10	0.00	0.00	7.41
	B	2.08	4.81	16.67	7.55	9.90	3.92	5.85	10.26
3	A	5.95	1.61	12.58	7.26	0.00	5.05	20.29	6.90
	B	8.65	12.31	5.19	2.94	0.00	9.01	10.56	5.64
Average		4.90	4.04	9.77	3.79	3.26	3.52	6.41	5.14

[a]Each value represents the percent of the total area of the right coronary artery affected by atherosclerotic lesions.

Table 15. Experiment 6434 - Summary of the effect of fat source, protein level and level of intake on percent lesions in left coronary artery

		Treatment							
Protein level, %		18				12			
Fat source		Soyoil		Tallow		Soyoil		Tallow	
Intake level		High	Low	High	Low	High	Low	High	Low
Replication	Block								
1	A	7.79[a]	7.74	18.85	5.47	14.16	9.68	5.56	8.70
	B	5.08	8.26	0.00	0.88	3.60	0.60	4.00	4.96
2	A	7.97	0.72	9.02	1.92	1.82	1.05	2.10	7.40
	B	1.89	8.20	26.12	3.50	11.57	5.03	5.63	20.40
3	A	4.01	2.00	10.79	9.02	2.52	0.00	14.20	10.94
	B	8.72	13.99	11.92	8.97	6.19	11.40	6.50	1.78
Average		5.91	6.82	12.78	4.96	6.64	4.63	6.33	9.03

[a]Each value represents the percent of the total area of the left coronary artery affected by atherosclerotic lesions.

Table 16. Experiment 6434 – Summary of the effect of fat source, protein level and level of intake on percent lesions in thoracic aorta

		Treatment							
Protein level, %		18				12			
Fat source		Soyoil		Tallow		Soyoil		Tallow	
Intake level		High	Low	High	Low	High	Low	High	Low
Replication	Block								
1	A	2.36[a]	1.01	26.12	1.64	0.00	15.38	2.83	3.52
	B	0.25	0.50	0.92	1.84	1.00	0.68	1.07	0.76
2	A	2.45	1.36	0.73	0.41	0.00	1.44	1.02	30.36
	B	0.45	0.30	1.47	0.53	0.28	0.52	1.18	17.52
3	A	1.86	0.00	0.43	0.54	1.78	0.50	2.26	1.08
	B	0.78	0.47	0.76	5.17	0.40	0.78	14.98	1.61
Average		1.52	0.61	5.07	1.69	0.58	3.22	3.89	9.14

[a]Each value represents the percent of the total area of the thoracic aorta affected by atherosclerotic lesions.

Table 17. Experiment 6434 - Summary of the effect of fat source, protein level and level of intake on percent lesions in abdominal aorta

		Treatment							
Protein level, %		18				12			
Fat source		Soyoil		Tallow		Soyoil		Tallow	
Intake level		High	Low	High	Low	High	Low	High	Low
Replication	Block								
1	A	8.31[a]	15.68	11.43	11.27	15.14	17.03	10.58	3.97
	B	3.25	3.01	6.63	16.17	2.15	9.02	5.58	1.88
2	A	4.85	5.47	4.72	2.80	3.43	20.35	4.26	18.30
	B	5.12	3.95	3.81	3.37	2.42	5.82	15.76	21.51
3	A	3.66	2.51	13.76	8.62	16.24	5.58	11.88	3.20
	B	10.93	2.27	4.06	4.89	5.53	2.00	79.97	7.95
Average		6.02	5.48	7.40	7.85	7.48	9.97	21.34	9.47

[a]Each value represents the percent of the total area of the abdominal aorta affected by atherosclerotic lesions.

and abdominal aorta are presented in Figures 16 to 20 and Tables 18 to 23. The analysis of variance plans and observed mean squares are presented in Appendix Tables 40 to 42.

The differences in serum cholesterol levels among samples taken at different times during the course of the experiment were statistically significant ($P < .01$). The serum cholesterol levels increased in a linear manner during the course of the experiment. The feeding of the diets containing tallow resulted in higher serum cholesterol levels than those observed in the pigs fed the diets containing soyoil; however, the difference was not statistically significant. The addition of cholesterol to the diets resulted in a significant ($P < .01$) increase in serum cholesterol levels over those observed in pigs fed the same diet treatments without the inclusion of cholesterol. Though the source of fat and cholesterol interaction was not significant, it was noted that the addition of cholesterol to the diet containing tallow resulted in higher serum cholesterol levels than did a similar cholesterol addition to the diet containing soyoil.

The feeding of the diets containing tallow resulted in a trend toward an increased incidence of lesions in the right and left coronary arteries over the incidence of lesions noted in the animals fed the diets containing soyoil. The addition of 1% cholesterol to the diets resulted in a significantly higher ($P < .01$) incidence of lesions in the pigs fed these diets over the incidence noted in animals fed similar diets but without the inclusion of cholesterol. The fat source and cholesterol interaction was not statistically significant; however, the addition of 1% cholesterol to the diet containing tallow resulted in a greater incidence of lesions than did a similar cholesterol addition to the diet containing soyoil.

Figure 16. Experiment 6512 - Effect of cholesterol addition to different fat sources on serum cholesterol levels

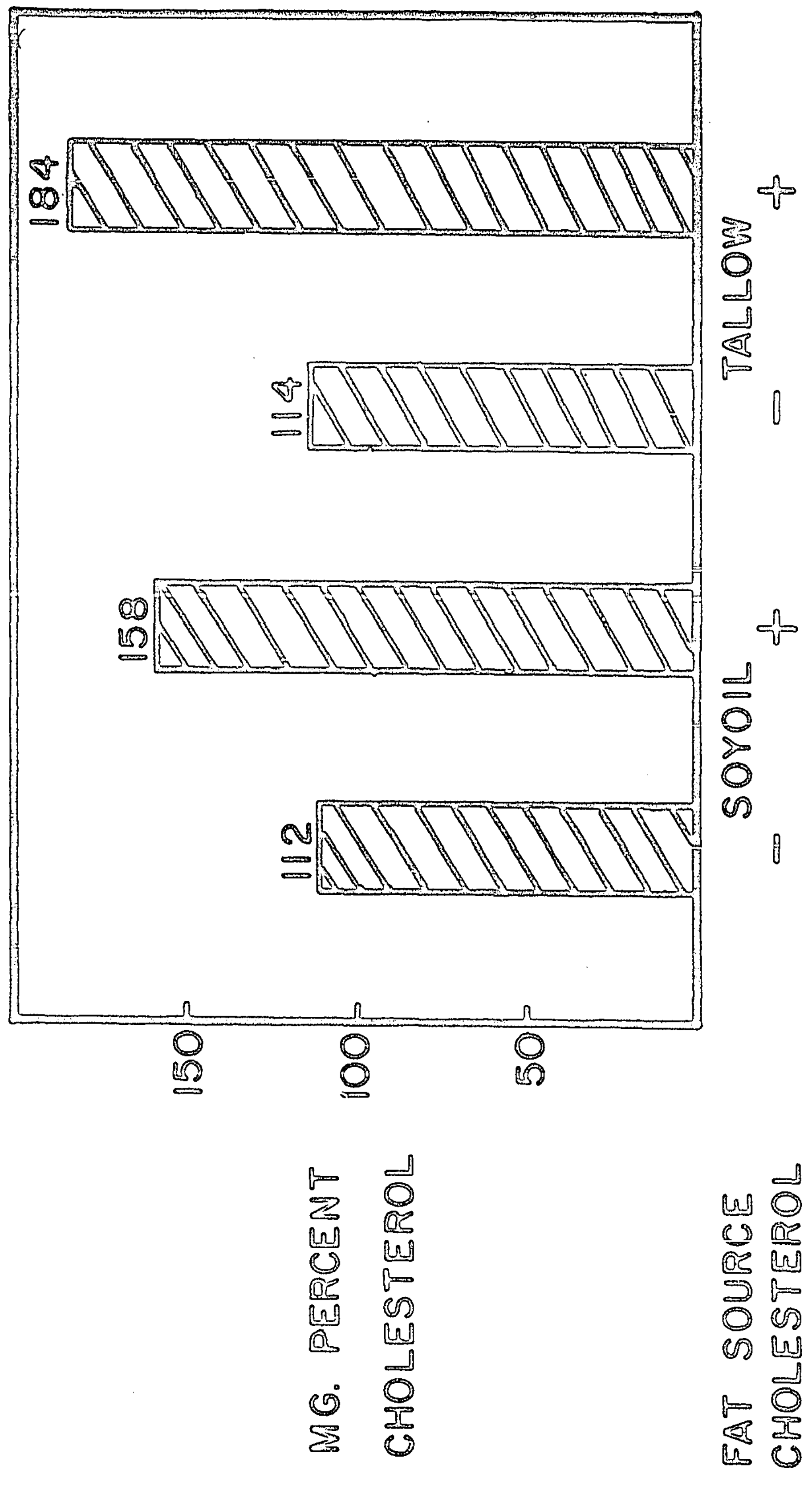

MG. PERCENT
CHOLESTEROL
150
100
50
112
158
114
184
SOYOIL
TALLOW
FAT SOURCE
CHOLESTEROL
−
+
−
+

Figure 17. Experiment 6512 - Effect of cholesterol addition to different fat sources on percent of the total area of the right coronary artery affected by lesions

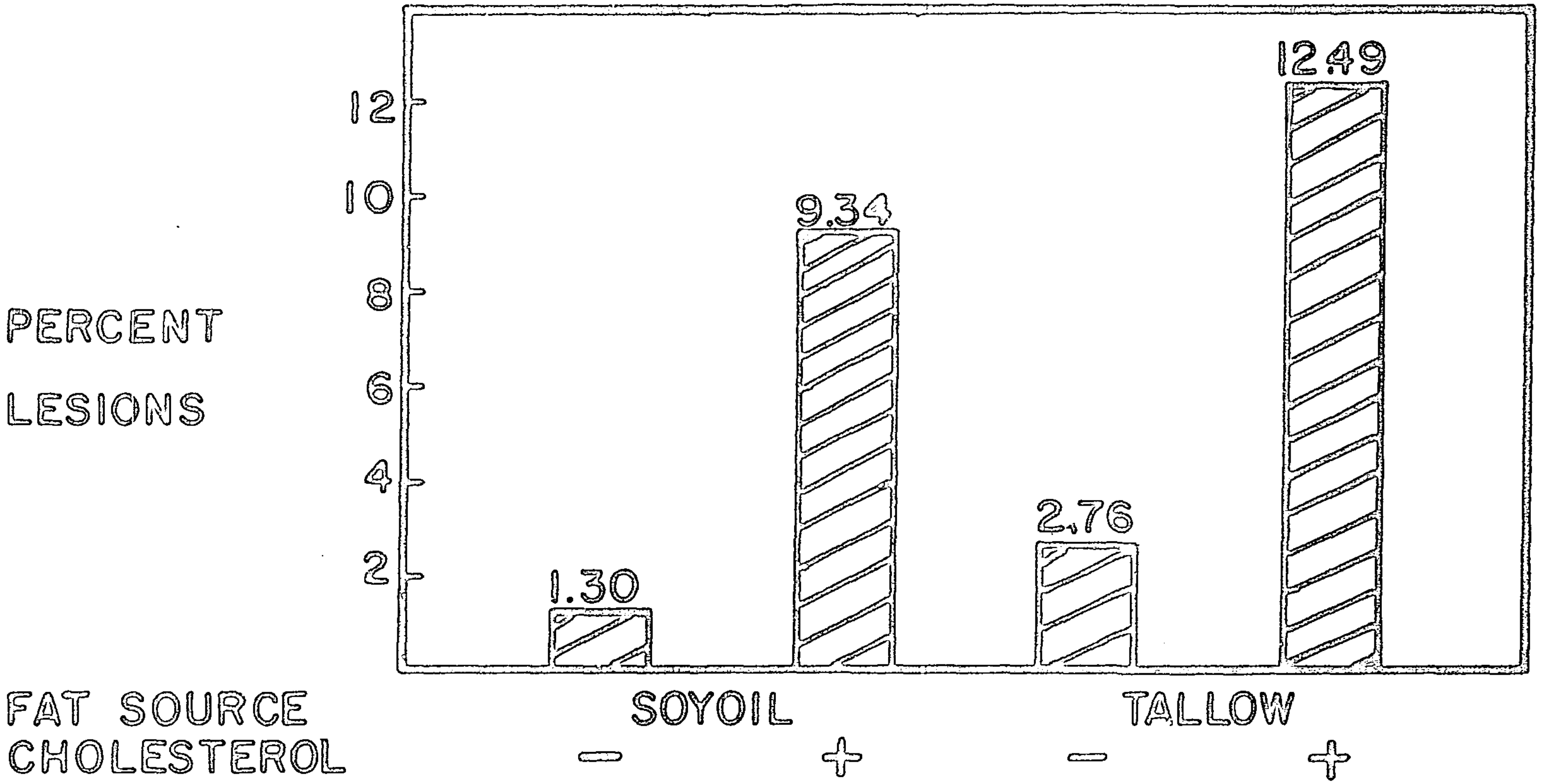

PERCENT
LESIONS
12
10
8
6
4
2
1.30
9.34
2.76
12.49
FAT SOURCE
CHOLESTEROL
SOYOIL
TALLOW
−
+
−
+

Figure 18. Experiment 6512 - Effect of cholesterol addition to different fat sources on percent of the total area of the left coronary artery affected by lesions

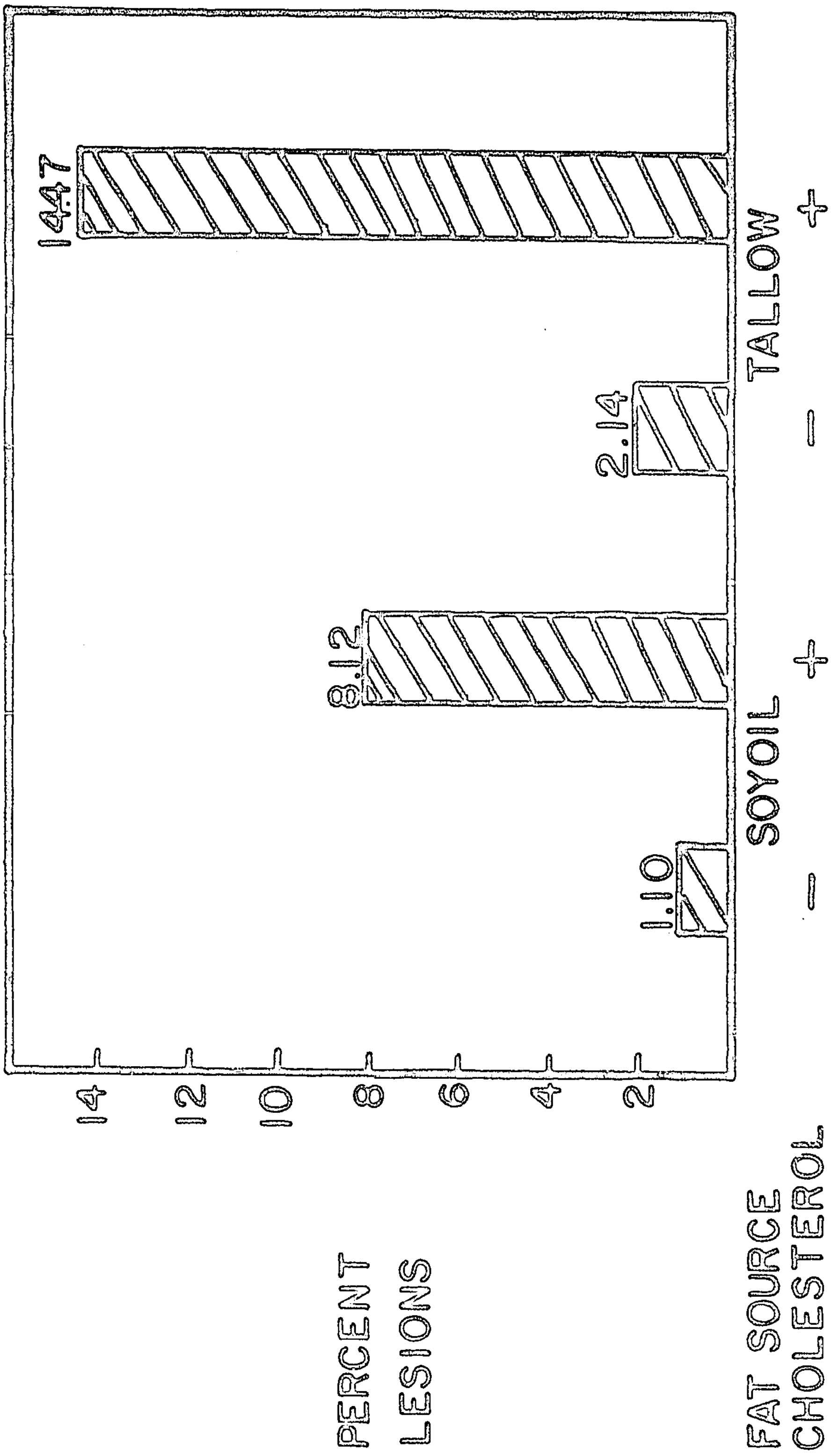

PERCENT LESIONS
14
12
10
8
6
4
2
1.10
8.12
2.14
14.47
FAT SOURCE
SOYOIL
TALLOW
CHOLESTEROL
–
+
–
+

Figure 19. Experiment 6512 - Effect of cholesterol addition to different fat sources on percent of the total area of the thoracic aorta affected by lesions

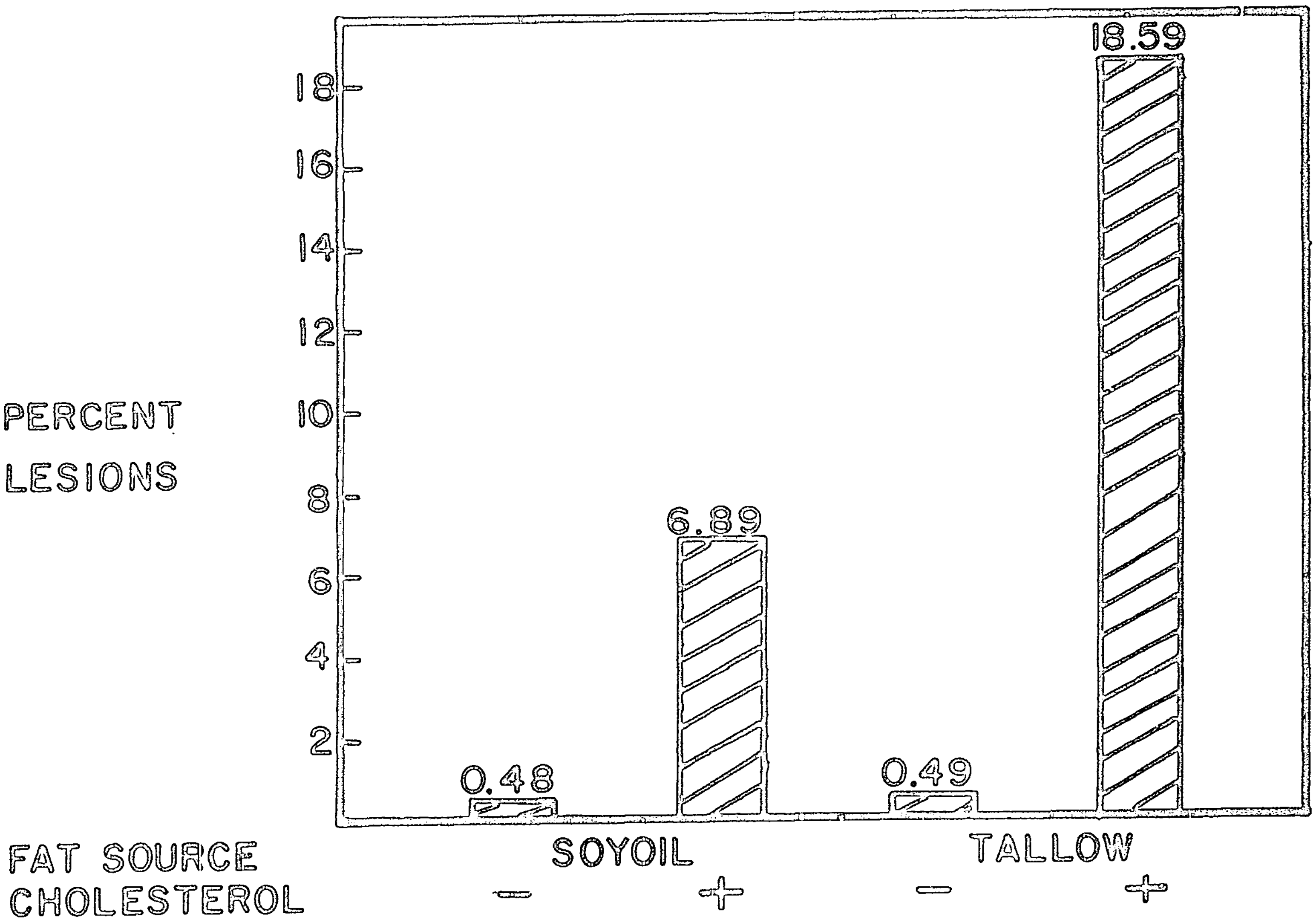

PERCENT
LESIONS
18
16
14
12
10
8
6
4
2
0.48
6.89
0.49
18.59
FAT SOURCE
CHOLESTEROL
SOYOIL
TALLOW
−
+
−
+

Figure 20. Experiment 6512 - Effect of cholesterol addition to different fat sources on percent of the total area of the abdominal aorta affected by lesions

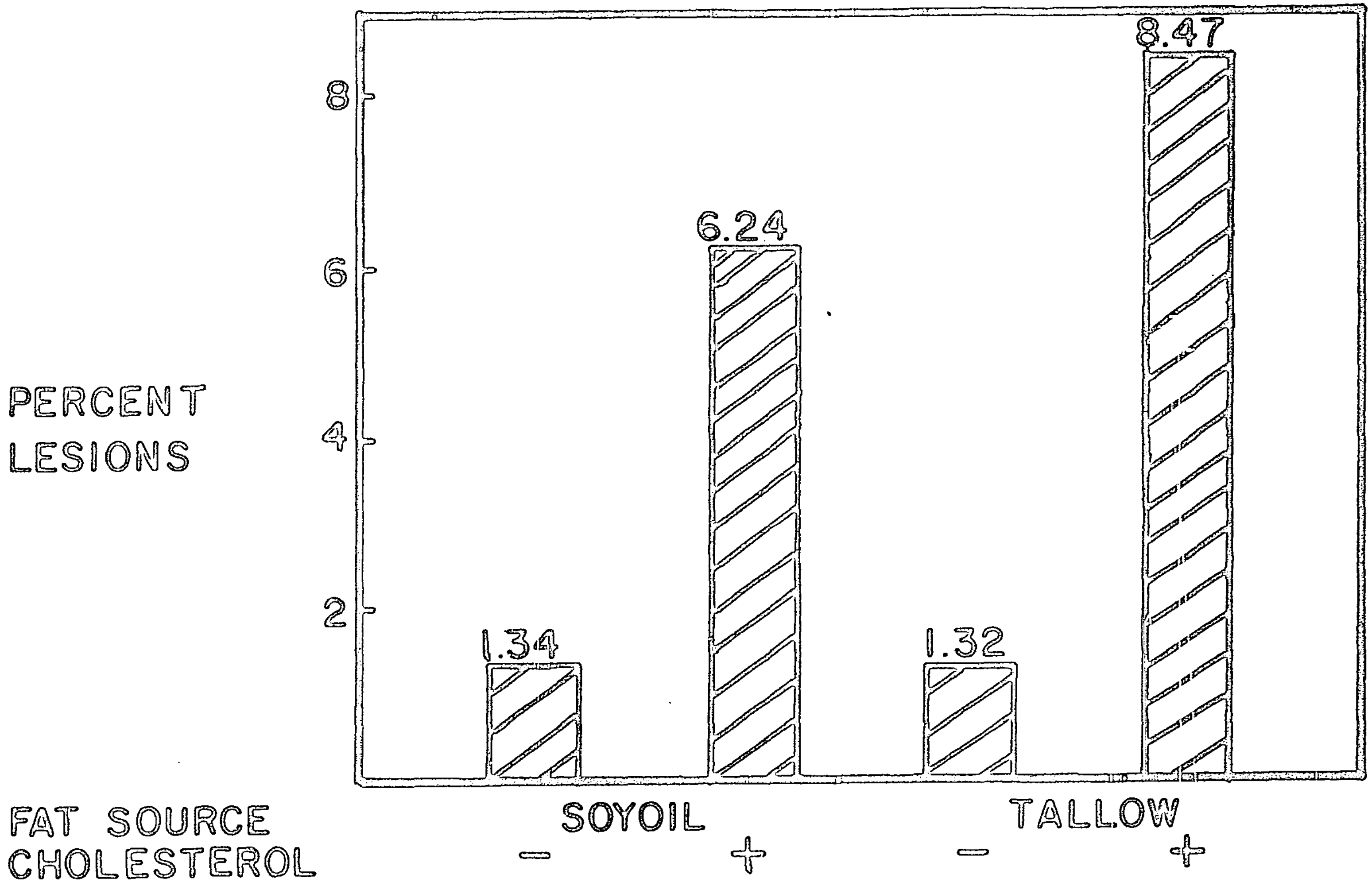

PERCENT LESIONS
8
6
4
2
8.47
6.24
1.34
1.32
FAT SOURCE
CHOLESTEROL
SOYOIL
TALLOW
−
+
−
+

Table 18. Experiment 6512 - Summary of the effect of cholesterol addition to different fat sources on serum cholesterol levels

	Treatment			
Fat source	Soyoil		Tallow	
Cholesterol, 1%	-	+	-	+
Time on experiment (wks.)				
0	106[a]	108	106	96
2	106	142	115	159
4	103	145	110	159
6	106	144	105	202
8	123	188	120	219
10	126	220	125	269
Average	112	158	114	184

[a]Each value represents the average for 12 pigs.

Table 19. Experiment 6512 - Summary of average daily food intake (Kg.)

	Treatment			
Fat source	Soyoil		Tallow	
Cholesterol, 1%	-	+	-	+
Replication				
1	2.67[a]	3.07	3.02	2.81
2	2.87	2.96	2.67	2.79
3	3.27	2.51	2.38	2.74
4	2.68	2.72	2.59	2.77
Average	2.87	2.82	2.66	2.78
Energy intake[b]	9439	9176	8818	9118

[a]Each value represents the average for three pigs.

[b]Kilocalories of metabolizable energy consumed per day.

Table 20. Experiment 6512 - Summary of effect of cholesterol addition to different fat sources on percent lesions in right coronary artery

Fat source	Treatment			
	Soyoil		Tallow	
Cholesterol, 1%	-	+	-	+
Replication				
1	0.74[a]	5.00	4.41	5.40
	1.90	6.25	9.26	6.99
	0.00	18.10	3.03	6.20
2	0.71	5.00	1.65	10.74
	0.00	6.54	0.00	7.02
	1.39	2.38	0.00	4.88
3	1.10	1.65	3.00	4.00
	8.80	4.44	3.52	17.69
	0.00	7.25	3.10	7.56
4	0.00	2.96	4.14	30.23
	0.63	34.15	1.00	24.15
	0.31	18.33	0.00	25.00
Average	1.30	9.34	2.76	12.49

[a]Each value represents the percent of the total area of the right coronary artery affected by atherosclerotic lesions.

Table 21. Experiment 6512 - Summary of the effect of cholesterol addition to different fat sources on percent lesions in left coronary artery

	Treatment			
Fat Source	Soyoil		Tallow	
Cholesterol, 1%	-	+	-	+
Replication				
1	0.00[a]	4.92	7.14	1.82
	0.74	0.00	5.47	2.27
	0.86	6.25	3.55	9.60
2	0.00	3.15	0.00	29.20
	0.00	17.46	0.40	11.98
	0.00	8.09	0.00	10.62
3	0.00	0.00	2.05	12.17
	5.36	1.28	2.03	19.67
	0.00	8.11	0.00	5.77
4	3.20	7.02	3.51	35.77
	3.02	11.50	1.55	10.87
	0.00	29.70	0.00	23.93
Average	1.10	8.12	2.14	14.47

[a]Each value represents the percent of the total area of the left coronary artery affected by atherosclerotic lesions.

Table 22. Experiment 6512 - Summary of the effect of cholesterol addition to different fat sources on percent lesions in thoracic aorta

Fat source	Treatment: Soyoil		Treatment: Tallow	
Cholesterol, 1%	-	+	-	+
Replication				
1	0.16[a]	1.36	0.00	10.96
	0.83	23.64	0.00	0.76
	0.27	5.34	0.63	1.33
2	0.58	0.10	0.54	47.24
	0.27	1.40	0.41	38.50
	0.35	0.48	0.88	4.42
3	0.42	0.00	0.52	3.22
	0.92	36.05	0.87	73.16
	0.52	8.77	0.51	0.00
4	0.70	0.55	0.26	11.00
	0.23	3.78	0.43	12.70
	0.55	1.16	0.85	19.77
Average	0.48	6.89	0.49	18.59

[a]Each value represents the percent of the total area of the thoracic aorta affected by atherosclerotic lesions.

Table 23. Experiment 6512 - Summary of the effect of cholesterol addition to different fat sources on percent lesions in abdominal aorta

	Treatment			
Fat source	Soyoil		Tallow	
Cholesterol, 1%	-	+	-	+
Replication				
1	0.00[a]	4.49	0.34	8.92
	0.00	6.52	0.00	1.60
	0.00	6.31	0.28	2.08
2	0.00	6.85	0.32	12.58
	0.55	2.82	0.00	12.57
	0.00	1.33	0.74	0.91
3	5.44	0.16	0.00	4.41
	1.39	10.31	5.19	15.56
	0.00	19.73	1.44	0.20
4	3.49	1.65	1.75	8.60
	4.40	8.40	3.54	11.49
	0.77	6.30	2.28	22.75
Average	1.34	6.24	1.32	8.47

[a]Each value represents the percent of the total area of the abdominal aorta affected by atherosclerotic lesions.

The pigs had approximately the same incidence of lesions in the left coronary artery as in the right coronary artery.

The incidence of lesions in the thoracic and abdominal aortas resulting from the dietary treatments exhibited the same pattern as that described for the right and left coronary arteries. The pigs had a greater incidence of lesions in the thoracic aorta than in the abdominal aorta.

GENERAL DISCUSSION

In this study three separate experiments involving 144 pigs were conducted to investigate dietary effects on the production of atherosclerosis. The dietary factors studied were fat source, level of intake, protein level and the effect of the addition of cholesterol to the diet. Serum cholesterol levels and the percent of the total area of the right and left coronary arteries, and the thoracic and abdominal aortas affected by atherosclerotic lesions were used as criteria for the incidence of the disease.

Lesions were present as fatty streaks and raised plaques, and the area of the vessel covered by these was used in estimating the percent of the total area involved with atherosclerotic lesions. No attempt was made to establish whether or not there was a relationship between these two types of lesions. In general it was observed that the fatty streaks and plaques frequently merged with each other. This finding would support the theory that the plaque may be an advanced stage of development of the fatty streak. Skold (1962) noted a similar finding in his investigation as well as isolated fatty streaks and plaques.

Sites and General Degree of Involvement

Lesions in the coronary arteries were noted and were generally confined to the first few centimeters of the right coronary artery and of the two main divisions of the left coronary artery. Similar findings have been reported by Skold (1962), Downie et al. (1963) and French et al. (1963). In Experiment 6415 it was noted that the main branch of the right coronary arteries had a greater incidence of lesions than the two main branches of the left coronary arteries; however, in Experiment 6434, the incidence was reversed, that is, the left coronary arteries had a greater percent of their total area involved than did the right coronaries. In Experiment 6512 a similar percent of area involvement was noted. The finding of a greater involvement of the two main branches of the left coronary than of the main branch of the right is in agreement with Duff and McMillan (1951) and Levene (1956) who report a greater incidence at this site in man.

Gubner and Ungerleider (1949) state that the intramural systolic pressure in the right ventricle and auricle is lower than in the left ventricle and does not cause any considerable retardation of blood flow. These authors state that it is for this reason that atherosclerosis is less marked in the right coronary artery which supplies the right chambers. Findings in these three trials conducted would not substantiate this view since in only one of the trials was the left artery found to have the greater incidence of disease. It would thus appear that a factor other than intramural systolic pressure is involved.

A greater amount of plaque formation was noted in the abdominal aortas than in the thoracic aortas. This finding is in agreement with Schwartz and Mitchell (1962) who observed similar findings in man, and Skold (1962) who observed similar findings in pigs. The plaques were more prevalent around the bifurcation of the aorta and the external and internal iliac arteries.

In Experiments 6415 and 6434, the abdominal aorta was found to have a greater percent of total area involvement than the thoracic, whereas in Experiment 6512 the thoracic was found to have the greater total area involvement of the two. Bragdon _et al._ (1957) reported that the lesions of experimentally produced atherosclerosis in swine were more prevalent in the thoracic aorta. Downie _et al._ (1963) reported similar findings to those of Bragdon _et al._ (1957) but noted that with age the distribution of atherosclerosis became more pronounced in the abdominal aorta. Experiment 6512 lasted a total time of 96 days, whereas in Experiments 6415 and 6434 the time was 140 and 257 days respectively, so the time factor could possibly account for the findings obtained. The overall findings in the three trials of the lesions in the thoracic and abdominal aortas are similar to the findings of Stephenson _et al._ (1962) who noted that in man the greatest concentrations of lesions were in the abdominal aorta and common iliac arteries rather than in the thoracic aorta.

Effect of Fat Source

In the comparison of soyoil and tallow as the fat source in the diet it was noted, though it was not always statistically significant, that tallow diets resulted in higher serum cholesterol levels. In general, the pigs in Experiments 6434 and 6512 which received the tallow diets had a greater incidence of lesions than did those fed the soyoil diets. In Experiment 6415 however, lesions were more prevalent in the coronary arteries but not in the thoracic and abdominal aortas of the pigs receiving the diets supplemented with soyoil. Neither of these findings were statistically significant and there appears to be no obvious reason as to why soyoil produced this effect in Experiment 6415 and not in the other two experiments. Since the caloric intake per day from the fat sources was maintained fairly constant and the above effects were noted, it would appear that the type and not the quantity of the fat source was responsible for the results observed. The findings in Experiments 6434, 6512 and 6415 in part, are consistent with work reported by Bragdon et al. (1957), Peifer and Lundberg (1957), Rowsell et al. (1958) and Barnes et al. (1959a) who used either serum cholesterol or post mortem examination for evaluation of the atherosclerotic status of the animal, and concluded that saturated fats give rise to a greater incidence of atherosclerosis than do unsaturated fats.

Barnes et al. (1961) and Gresham et al. (1964) however found no evidence from their studies that the type of fat in the diet influenced the degree of atherosclerosis. Reiser et al. (1959) however reported that the only typical atheromatous lesion found in their trial resulted from the ingestion of unsaturated dietary fat plus cholesterol, and that plasma cholesterol levels were influenced more by unsaturated than by saturated fat.

Gresham et al. (1964) investigated the possibility that an essential fatty acid deficiency was involved in the production of atherosclerosis but found no evidence to suggest that the atherosclerotic lesions which they observed were caused by a dietary deficiency or relative deficiency of essential fatty acids.

Peifer and Lundberg (1957) reported that, from their studies on the effect of the influence of specific fatty acids on the development of atherosclerosis, pigs receiving ethyl linolenate had only a small amount of plaque formation in their aortas and pigs receiving ethyl linoleate had more extensive aortic deposits, while those receiving oleate or saturated esters had very extensive deposits over the entire surface of the thoracic aorta.

Effect of Energy Intake

The effect of high and low energy intake levels on the development of atherosclerosis was studied in Experiments 6415 and 6434. A trend for an increased incidence of lesions as the caloric intake was restricted was noted in Experiment 6415; however, this effect was only significant in the case of the abdominal aorta. In the case of the right coronary artery in Experiment 6415 it was noted that the animals on the high intake level had the greater incidence of disease. On the other hand, in Experiment 6434 there was a trend for the animals on the high energy intake, with the exception being in the case of the thoracic aorta, to have the greater incidence of lesions. Serum cholesterol levels for the overall high and low energy intake levels in each experiment were similar though in Experiment 6415 a significant fat source and level of feeding interaction was noted.

Peifer and Lundberg (1958 and 1962) reported that on the basis of serum cholesterol evaluation they found no effects attributable to the total caloric intake _per se_; however, increasing the caloric intake from the fat source elevated the serum cholesterol level. The observations in these experiments would agree with the findings of Peifer and Lundberg (1958 and 1962) to the extent that it appears to be the caloric intake from the fat source itself which is important. Only in Experiment 6434 was an increase in the incidence of disease observed from the high intake level.

The incidence of disease as related to level of feed intake was inconsistent and did not appear related to serum cholesterol levels. It would appear that the evaluation of the atherosclerotic state on the basis of serum cholesterol level alone could be misleading, since it would appear that serum cholesterol elevation is not necessary for development of the disease. Downie *et al.* (1963) also conclude from evaluation of their own and other studies that whatever the role of blood lipid is in the development of atherosclerosis, its elevation is not necessary for development of the disease. Hays *et al.* (1963) reported that total caloric intake could significantly influence the serum cholesterol levels; however, the differences in level of intake were much greater and would suggest that great excesses of energy intake may increase serum cholesterol levels.

Effect of Protein Level

The effect of 12% versus 18% protein levels was studied in Experiment 6434. A trend was observed, though it was not statistically significant, in that the low protein diets resulted in elevation of the serum cholesterol levels and a greater incidence of lesions in the thoracic and abdominal aortas. The high protein diets on the other hand resulted in a greater involvement of the right and left coronary arteries. This observed trend does not correspond with the report of Barnes *et al.* (1959a) who studied the effects of 13.7% versus 4.9% protein level diets and found that serum cholesterol levels were not influenced by protein level. Similar findings were reported by Moreland _et al_. (1963). Barnes _et al_. (1959b) however, on comparing 16% versus 9% protein level diets did find serum cholesterol elevation with the low protein diet. Hays _et al_. (1963) reported a significant protein and protein and energy interaction effect in gestating pigs. High protein intake elevated the serum cholesterol level and the effect was greater with the higher level of energy intake. This report of Hays _et al_. (1963) is therefore not consistent with the results obtained in this study or the results published by Barnes _et al_. (1959a and 1959b) or Moreland _et al_. (1963).

Effect of Added Cholesterol

The addition of cholesterol to either soyoil or tallow supplemented diets in Experiment 6512 significantly increased the serum cholesterol level and incidence of lesions in all blood vessels examined as compared with that found in pigs on similar diets but without the addition of cholesterol. This finding is in general agreement with the reports of Downie _et al_. (1963) and Moreland _et al_. (1963).

In the work reported by Reiser _et al_. (1959) the unsaturated fat increased the apparent absorption of cholesterol whereas the saturated fat depressed the absorption. The findings in this trial would not support the work of Reiser _et al_. (1959), since it was noted, though it was not statistically significant, that on cholesterol addition to the diet the saturated fat source (tallow) resulted in a greater incidence of lesions and also higher serum cholesterol levels in animals fed this diet as opposed to those fed a diet similarly supplemented with an unsaturated fat source, soyoil.

In work with cholesterol supplementation using rabbits, Friedman and Byers (1965) demonstrated that quantities of cholesterol as low as 50 mg. per 100 gm. of food, which were insufficient to alter the serum concentration or to promote the deposition of excess lipid and cholesterol in the intact aorta, were observed, nevertheless, to accumulate in great excess in an experimentally-induced arterial plaque. These authors suggest that dietary derived cholesterol provides a heightened atherogenic potential to any arterial area in which hyperplasia is in process. This finding, as suggested by Friedman and Byers (1965), would call for reappraisal of the view such as expressed by the report of the Central Committee for Medical and Community Program of the American Heart Association (1961) that cholesterol in the human diet may be of only secondary pathogenetic importance.

Kritchevsky (1958) lists the cholesterol content of beef tallow as 75-140 mg. per 100 gm. of tallow and the U.S.D.A. Composition of Foods Handbook (1963) lists the cholesterol content of lard and other animal fat as 95 mg. per 100 gm. fat. The cholesterol content of animal fat when looked upon in the light of the findings of Friedman and Byers (1965) should call for reappraisal of the many reports on the effect of plant source fat as opposed to animal source fat in the diets. This point would seem to be important since, according to the report to the Council on Foods and Nutrition of the American Medical Association (1963) on the regulation of dietary fat, the concentration of fat calories in diets habitually ingested by Americans is about 40% of the total calories. About two-thirds of the total fat is of animal origin; the remainder comes from vegetable sources. The fact that Barnes *et al.* (1961) found similar serum cholesterol values in animals fed partially hydrogenated soybean oil and corresponding natural plant fat mixtures would suggest that the cholesterol content of animal fat may be equally as important as the degree of saturation.

Effect of Time after Feeding on Serum Cholesterol

The effect of time of sampling after feeding on serum cholesterol levels was studied in Experiment 6434, samples being taken at 2, 4 and 6 hours after feeding. The serum cholesterol values increased significantly in a linear manner with time. When the differences between treatments were compared at each sampling time with the corresponding serum cholesterol values obtained after a 16-hour fast and just prior to feeding, it was noted that differences were quite similar regardless of sampling time. This would suggest that treatment effects are no less evident in the fasted than in the non-fasted animal and hence that bleeding after a 16-hour fast was of no significance in the interpretation of the data.

SUMMARY

Three trials involving a total of 144 pigs were conducted to investigate dietary effects on the production of atherosclerosis. In trial 1 either soyoil or tallow was incorporated at a level of 15% in diets containing 14% protein, which were fed such that pigs consumed either 7245 or 5016 kilocalories of metabolizable energy daily. In trial 2 either soyoil or tallow was incorporated at a 15% level in diets containing 12% or 18% protein, which were fed such that pigs consumed either 6768 or 4811 kilocalories of metabolizable energy daily. In trial 3 soyoil or tallow was incorporated at a level of 5% in diets containing 14% protein. The diets in trial 3 were offered *ad libitum* with and without the inclusion of 1% cholesterol.

The criteria used for estimation of the incidence of atherosclerosis were serum cholesterol levels and the atherosclerotic lesions in the aorta and cardiac arteries.

In the coronary arteries the lesions were generally confined to the first few centimeters of the right coronary and of the two main branches of the left coronary. In trials 1 and 2 the incidence of lesions was greater in the abdominal aorta than in the thoracic, whereas in trial 3 the thoracic had the greater incidence. A greater incidence of plaque formation was noted in the abdominal aorta than in the thoracic aorta, with the most common site of occurrence being around the bifurcation of the aorta and the external and internal iliac arteries.

Animals receiving the diets containing tallow generally had the higher serum cholesterol levels. This finding was not always statistically sig-

nificant. In trials 2 and 3 feeding the diets containing tallow resulted in a trend toward an increased incidence of lesions. In trial 1 the same trend was noted, with the pigs receiving the diets supplemented with tallow having the greater incidence of lesions in the thoracic and abdominal aortas; however, the coronary arteries of the pigs receiving the diets supplemented with soyoil had the greater incidence of lesions.

The effect of the level of energy intake produced inconsistent results. Serum cholesterol levels were unaffected by the level of intake. In trial 1 there was a trend, the right coronary artery being an exception, for an increased incidence of lesions at the low caloric intake levels. This energy effect was significant only in the case of the abdominal aorta. On the other hand in trial 2 there was a trend, the thoracic aorta being an exception, for the animals on the high caloric intake levels to have the greater incidence of lesions.

The feeding of diets containing 12% protein resulted in slightly elevated serum cholesterol levels and a greater incidence of lesions in the thoracic and abdominal aortas than did the feeding of diets containing 18% protein. The high protein diets resulted in a greater incidence of lesions in the right and left coronary arteries than did the low protein diets.

Cholesterol addition to the diet resulted in a significant increase in serum cholesterol levels and incidence of lesions as compared with pigs on similar diets without cholesterol addition. The feeding of diets containing tallow and supplemented with cholesterol resulted in a slightly greater elevation of serum cholesterol levels and incidence of lesions than did the feeding of similar diets containing soyoil and supplemented with cholesterol.

Treatment effects on serum cholesterol levels were found to be no less evident in fasted than in the non-fasted animals.

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APPENDIX

Table 24. Experiment 6415 - Composition of the experimental diets[a]

Ingredient	Tallow	Soyoil	Control
	%	%	%
Ground yellow corn	63.95	63.95	82.20
Tallow (stabilized, edible)	15.00	---	---
Soybean oil (degummed)	---	15.00	---
Solvent soybean meal (50% protein)	16.45	16.45	13.25
Vitamin-antibiotic premix[b]	2.00	2.00	2.00
Calcium carbonate (38% calcium)	0.70	0.70	0.85
Dicalcium phosphate (26% calcium, 18% phosphorus)	1.30	1.30	1.10
Iodized salt	0.50	0.50	0.50
Trace mineral premix[c]	0.10	0.10	0.10
Total	100.00	100.00	100.00

[a]Calculated analysis presented in Table 25.

[b]Composition presented in Table 30.

[c]Composition presented in Table 31.

Table 25. Experiment 6415 - Calculated analysis of the experimental diets

Item		Tallow	Soyoil	Control
Protein	Percent	14.0	14.0	14.0
Metabolizable energy	Kcal./kg.	3742	3665	3095
Calcium	Percent	.65	.65	.65
Phosphorus	Percent	.50	.50	.49
Vitamin A	I.U./kg.	3201.0	3201.0	3606.0
Vitamin D_2	I.U./kg.	662.3	662.3	662.3
Riboflavin	Mg./kg.	5.5	5.5	5.7
Pantothenic acid	Mg./kg.	14.1	14.1	14.5
Niacin	Mg./kg.	37.0	37.0	40.0
Choline	Mg./kg.	778.8	778.8	770.0
Vitamin B_{12}	Mcg./kg.	22.0	22.0	22.0
Chlortetracycline	Mg./kg.	44.0	44.0	44.0
Ethoxyquin	Mg./kg.	74.8	74.8	74.8

Table 26. Experiment 6434 - Composition of the experimental diets[a]

Ingredient	18% protein		12% protein	
	Soyoil	Tallow	Soyoil	Tallow
	%	%	%	%
Ground yellow corn	25.50	25.50	39.70	39.70
Soybean oil (degummed)	15.00	---	15.00	---
Tallow (stabilized, edible)	---	15.00	---	15.00
Ground corn cobs (dehydrated)	25.00	25.00	25.00	25.00
Solvent soybean meal (50% protein)	30.05	30.05	15.65	15.65
Vitamin-antibiotic premix[b]	2.00	2.00	2.00	2.00
Calcium carbonate (38% calcium)	0.60	0.60	0.45	0.45
Dicalcium phosphate (26% calcium, 18% phosphorus)	1.25	1.25	1.60	1.60
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral premix[c]	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00

[a]Calculated analysis presented in Table 27.

[b]Composition presented in Table 30.

[c]Composition presented in Table 31.

Table 27. Experiment 6434 - Calculated analysis of the experimental diets

Item		18% protein		12% protein	
		Soyoil	Tallow	Soyoil	Tallow
Protein	Percent	18.0	18.0	12.0	12.0
Metabolizable energy	Kcal./kg.	2869	2948	2983	3062
Calcium	Percent	.65	.65	.65	.65
Phosphorus	Percent	.50	.50	.50	.50
Vitamin A	I.U./kg.	2362.4	2362.4	2674.8	2674.8
Vitamin D_2	I.U./kg.	662.3	662.3	662.3	662.3
Riboflavin	Mg./kg.	5.5	5.5	5.3	5.3
Pantothenic acid	Mg./kg.	14.1	14.1	12.9	12.9
Niacin	Mg./kg.	31.8	31.8	31.8	31.8
Choline	Mg./kg.	1001.9	1001.9	652.5	652.5
Vitamin B_{12}	Mcg./kg.	22.0	22.0	22.0	22.0
Chlortetracycline	Mg./kg.	44.0	44.0	44.0	44.0
Ethoxyquin	Mg./kg.	124.3	124.3	124.3	124.3

Table 28. Experiment 6512 - Composition of the experimental diets[a]

Ingredient	Controls Soyoil	Controls Tallow	1% cholesterol Soyoil	1% cholesterol Tallow
	%	%	%	%
Ground yellow corn	75.85	75.85	74.60	74.60
Soybean oil (degummed)	5.00	---	5.00	---
Tallow (stabilized, edible)	---	5.00	---	5.00
Cholesterol	---	---	1.00	1.00
Solvent soybean meal (50% protein)	14.55	14.55	14.80	14.80
Vitamin-antibiotic premix[b]	2.00	2.00	2.00	2.00
Calcium carbonate (38% calcium)	0.80	0.80	0.80	0.80
Dicalcium phosphate (26% calcium 18% phosphorus)	1.20	1.20	1.20	1.20
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral premix[c]	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00

[a]Calculated analysis presented in Table 29.

[b]Composition presented in Table 30.

[c]Composition presented in Table 31.

Table 29. Experiment 6512 - Calculated analysis of the experimental diets

Item		Controls		1% cholesterol	
		Soyoil	Tallow	Soyoil	Tallow
		%	%	%	%
Protein	Percent	14.1	14.1	14.1	14.1
Metabolizable energy	Kcal./kg.	3289	3315	3254	3280
Calcium	Percent	.66	.66	.66	.66
Phosphorus	Percent	.51	.51	.50	.50
Vitamin A	I.U./kg.	3469.0	3469.0	3443.0	3443.0
Vitamin D_2	I.U./kg.	662.3	662.3	662.3	662.3
Riboflavin	Mg./kg.	5.6	5.6	5.6	5.6
Pantothenic acid	Mg./kg.	14.5	14.5	14.4	14.4
Niacin	Mg./kg.	39.1	39.1	38.9	38.9
Choline	Mg./kg.	781.0	781.0	781.0	781.0
Vitamin B_{12}	Mcg./kg.	22.0	22.0	22.0	22.0
Chlortetracycline	Mg./kg.	44.0	44.0	44.0	44.0
Ethoxyquin	Mg./kg.	124.3	124.3	124.3	124.3

Table 30. Amounts of vitamins, antibiotic and antioxidant added per kilogram of complete diet in all experiments

Item		
Vitamin A	I.U.	1760
Vitamin D_2	I.U.	662.3
Riboflavin	Mg.	4.4
Calcium pantothenate	Mg.	8.8
Niacin	Mg.	19.8
Choline chloride	Mg.	22.0
Vitamin B_{12}	Mcg.	22.0
Chlortetracycline	Mg.	44.0
Ethoxyquin[a]	Mg.	124.3

[a]In Experiment 6415 ethoxyquin was added at the rate of 74.8 mg./kg.

Table 31. Composition of trace mineral premix[a]

Element	Percent in premix	Parts per million added to ration
Iron	7.000	70.4
Copper	0.475	4.8
Cobalt	0.166	1.7
Manganese	5.680	56.8
Zinc	8.100	81.0
Potassium	0.750	7.5
Calcium	5.280	--

[a]Added at level of 0.10 percent of ration.

Table 32. Experiment 6415 - Analysis of variance plan and observed mean squares for serum cholesterol levels

Source of variation	d.f.	Mean squares
Replication	7	3744**
Sex	1	93
Replication/sex	6	4352**
Treatment	5	11702**
Fat source	2	21403**
Control *vs.* added fat	1	26477**
Soyoil *vs.* tallow	1	16329**
High intake *vs.* low intake	1	0
Fat source X level of feeding	2	7853*
Time on experiment	10	26824**
Replication X treatment	35	1609**
Treatment X time on experiment	50	419**
Replication X time on experiment	70	156
Replication X treatment X time on experiment	278	254
Total	455	1125

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.

Table 33. Experiment 6415 - Analysis of variance plan and observed mean squares for percent lesions in right coronary artery

Source of variation	d.f.	Mean squares
Replication	6	11.2444
Treatment	5	5.9146
Fat source	2	12.2806
Control *vs.* added fat	1	5.9467
Soyoil *vs.* tallow	1	18.6146
High intake *vs.* low intake	1	3.9560
Fat source X level of feeding	2	.5280
Error	30	14.6253
Total	41	13.0682

Table 34. Experiment 6415 - Analysis of variance plan and observed mean squares for percent lesions in left coronary artery

Source of variation	d.f.	Mean squares
Replication	7	45.9154**
Sex	1	153.4390**
Replication/sex	6	27.9948*
Treatment	5	14.5641
Fat source	2	29.3329
Control *vs.* added fat	1	39.3216*
Soyoil *vs.* tallow	1	19.3442
High intake *vs.* low intake	1	11.7414
Fat source X level of feeding	2	1.2066
Error	35	9.3625
Total	47	15.3599

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.

Table 35. Experiment 6415 - Analysis of variance plan and observed mean squares for percent lesions in the thoracic and abdominal aortas

Source of variation	d.f.	Mean squares	
		Thoracic	Abdominal
Replication	7	2.5193	8.1592
Sex	1	1.6800	4.4590
Replication/sex	6	2.6592	8.7760
Treatment	5	1.1165	31.1411*
Fat source	2	.3450	19.0240
Control *vs.* added fat	1	.3700	.3117
Soyoil *vs.* tallow	1	.3200	37.7363
High intake *vs.* low intake	1	3.1314	59.9203*
Fat source X level of feeding	2	.8805	28.8685
Error	35	1.2200	9.5016
Total	47	1.4025	11.6972

*Statistical significance at P = 0.05 or less.

Table 36. Experiment 6434 - Analysis of variance plan and observed mean squares for serum cholesterol levels

Source of variation	d.f.	Mean squares
Blocks	5	5501
Replication	2	2878
Blocks/replication	3	7250
Sex	1	11000
Blocks/replication and sex	2	5375
Treatment	7	6210
Protein level	1	9815
Feeding level	1	33
Fat source	1	19647
Protein level X feeding level	1	6517
Protein level X fat source	1	110
Feeding level X fat source	1	1754
Protein level X fat source X feeding level	1	5591
Time on Experiment (Time)	16	6116
Block X treatment	35	2501
Replication X treatment	14	4890
Block/Replication X treatment	21	908
Time X block	80	308
Time X replication	32	268
Time X block/replication	48	334
Time X Treatment	112	206
Time X block X treatment	560	187
Time X replication X treatment	224	239
Time X block/replication X treatment	336	152
Total	815	502

Table 37. Experiment 6434 - Analysis of variance plan and observed mean squares for bleeding time after feeding effect on serum cholesterol levels

Source of variation	d.f.	Mean squares
Biweekly bleeding	3	3155**
Sets	5	1789*
Hours after feeding	2	2530*
Sets/hours after feeding	3	1295
Treatment	7	527
Protein level	1	491
Feeding level	1	36
Fat source	1	2768*
Protein level X feeding level	1	106
Protein level X fat source	1	69
Feeding level X fat source	1	62
Protein level X fat source X feeding level	1	156
Biweekly bleeding X treatment	21	115
Biweekly bleeding X set	15	495
Set X treatment	35	550
Biweekly bleeding X set X treatment	105	135
Total	191	342

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.

Table 38. Experiment 6434 - Analysis of variance plan and observed mean squares for percent lesions in right and left coronary arteries

Source of variation	d.f.	Mean squares	
		Right	Left
Blocks	5	48.7629	68.7797*
Replication	2	60.1636	4.8518
Sex	1	70.0350	150.2376*
Block/replication/sex	2	26.7261	91.9787*
Treatment	7	27.6846	42.0042
Protein level	1	13.0417	11.0592
Feeding level	1	46.0992	29.1408
Fat source	1	66.1291	62.1985
Protein level X feeding level	1	25.4917	43.2820
Protein level X fat source	1	.0161	.6395
Feeding level X fat source	1	33.3667	12.1003
Protein level X fat source X feeding level	1	9.6481	135.6095*
Block X treatment	35	18.2758	23.1544
Replication X treatment	14	26.8448	20.0278
Block/replication X treatment	21	12.5632	25.2388
Total	47	22.9204	30.8156

*Statistical significance at P = 0.05 or less.

Table 39. Experiment 6434 - Analysis of variance plan and observed mean squares for percent lesions in thoracic and abdominal aortas

Source of variation	d.f.	Mean squares	
		Thoracic	Abdominal
Blocks	5	38.5546	82.0774
Replication	2	14.6858	54.6998
Sex	1	3.9790	1.6317
Block/replication/sex	2	79.7113	149.6778
Treatment	7	50.2000	154.0078
Protein level	1	49.2075	346.7412
Feeding level	1	10.5844	67.3317
Fat source	1	147.7008	219.5213
Protein level X feeding level	1	108.4805	64.8908
Protein level X fat source	1	14.8074	69.1440
Feeding level X fat source	1	.0003	133.9006
Protein level X fat source X feeding level	1	20.6195	176.5251
Block X treatment	35	41.0508	143.5681
Replication X treatment	14	70.5096	173.2434
Block/replication X treatment	21	21.4117	123.7846
Total	47	42.1479	138.5814

Table 40. Experiment 6512 - Analysis of variance plan and observed mean squares for serum cholesterol levels

Source of variation	d.f.	Mean squares
Outcome groups	11	2495
Replication	3	3149
Outcome group/replication	8	2250
Sex	1	7792
Outcome group/replication and sex	7	1458
Time on experiment	5	38619**
Time on experiment X outcome group	55	944
Treatment	3	90387**
Fat source	1	13750
Cholesterol	1	246753**
Fat source X cholesterol	1	10659
Treatment X time on experiment	15	8238
Treatment X outcome group	33	3557
Treatment X replication	9	4002
Treatment X outcome group/replication	24	3390
Treatment X time on experiment X outcome group	165	812
Total	287	3201

**Statistical significance at P = 0.01 or less.

Table 41. Experiment 6512 - Analysis of variance plan and observed mean squares for percent lesions in right and left coronary arteries

Source of variation	d.f.	Mean squares	
		Right	Left
Outcome groups	11	59.6193	50.0539
Replication	3	159.5519	123.2029
Sex	1	40.8483	.7277
Outcome group/replication/sex	7	19.4727	25.7509
Treatment	3	339.8373*	457.4492**
Soyoil *vs.* tallow	1	63.8024	163.9472
Cholesterol *vs.* No cholesterol	1	947.1410**	1123.9449**
Fat source X cholesterol	1	8.5684	84.4556
Outcome group X treatment	33	43.3624	47.3749
Replication X treatment	9	85.4988	65.4572
Outcome group/replication X treatment	24	27.5612	40.5941
Total	47	66.0911	74.1769

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.

Table 42. Experiment 6512 - Analysis of variance plan and observed mean squares for percent lesions in thoracic and abdominal aortas

Source of variation	d.f.	Mean squares	
		Thoracic	Abdominal
Outcome groups	11	224.3374	23.1053
Replication	3	117.7896	36.8504
Sex	1	.0029	3.8533
Outcome group/replication/sex	7	302.0485	19.9648
Treatment	3	874.1063*	155.2186**
Soyoil *vs.* tallow	1	411.4309	14.7852
Cholesterol *vs.* no cholesterol	1	1800.6276**	435.7280**
Fat source X cholesterol	1	410.2605	15.1426
Outcome group X treatment	33	142.2510	19.5713
Replication X treatment	9	140.0518	17.0536
Outcome group/replication X treatment	24	143.0756	20.5154
Total	47	208.1769	29.0567

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.